Assessing Introgression between European Wildcats (*Felis silvestris silvestris*) and Domestic Cats (*Felis silvestris catus*)

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Summary

Introgression is an important issue in evolutionary biology. It is defined as the flow of genes between taxa through hybridization beyond the first generation. Introduced genes of a closely related taxon may serve as raw material for rapid adaptive evolutionary change. On the other hand, introgression could lead to reduced fitness in hybrids, i.e. outbreeding depression, if the newly mixed traits are maladapted to the environment or if, on the genomic level, co-adapted gene complexes are disrupted.

In conservation biology, introgression is often seen as a threat to genetic integrity by genome swamping and as especially relevant when hybridization is human-induced. This is the case for the European Wildcat (*Felis silvestris silvestris*), which is hybridizing with the domestic cat (*Felis silvestris catus*), potentially since over 2500 years. Hence, assessing the impact of introgression by monitoring free-ranging cat populations is crucial for wildcat conservation. However, introgression is difficult to detect. In wildcats, neither morphological nor genetic methods allowed accurate recognition of introgression so far.

In the present thesis, I aimed to provide the basic knowledge necessary to investigate the effects of introgression on wildcats by 1) developing genetic markers able to disclose introgression; 2) establishing a genotyping method for non-invasive samples that allow monitoring a wildcat population based on hair samples; 3) assessing the introgression rate in the wildcat population of the Jura region; and 4) describing hybridization patterns in wildcats from France, Germany and Switzerland.

Chapter 1 describes the development of single nucleotide polymorphism (SNP) markers, which allow reliable recognition of individual levels of introgression in wildcats, domestic cats and their admixed progeny. I first defined reference wildcats and domestic cats, based on the analysis of microsatellites, mitochondrial and Y-chromosome sequences, as well as morphological criteria. Secondly, I sequenced a selected small part of the genome of six reference wildcats and three domestic cats by high-throughput-sequencing. The comparison of the sequences revealed over 800'000 SNPs between both subspecies. I then selected 200 SNPs at which wildcats and domestic cats had differently fixed alleles and sequenced the regions on the genome containing these SNPs in an additional ten wildcats and 13 domestic cats, to validate the diagnostic value of these 200 SNPs. Using a Bayesian approach, I finally assessed the power of the 48 most differentiated SNP markers in determining the individual hybridization level of simulated hybrids until second generation of hybridization. This subset of SNPs allowed assessing the correct hybrid status with high accuracy, since 99.6% of the simulated individuals were assigned to the correct hybrid category. These SNP markers thus allow the reliable assessment of introgression levels in natural populations.

Non-invasive sampling is a common and efficient way to sample elusive populations like wildcats. But the limited quality and quantity of nuclear DNA extracted from non-invasively collected samples, like single hairs, is a challenge for accurate genotyping. **Chapter 2** shows how I optimized a SNP genotyping method to yield reliable genotypes of single hairs. I developed a 96.96 Fluidigm SNP genotyping array (SNP chip), based on the nuclear diagnostic SNPs described in chapter 1 and on published mitochondrial (mtDNA) SNPs. The SNP chip contained 75 nuclear SNP markers most differentiated between wildcats and domestic cats for recognition of the introgression level, 11 nuclear

markers and four mtDNA markers for recognition of individuals, four diagnostic mtDNA markers for maternal lineage assessment and two Y-linked markers for paternal lineage assessment and sex determination. Prior to genotyping, DNA extracted from single hairs was quantified with a cat specific real-time PCR assay. This step allows excluding hairs from species other than *Felis silvestris* and hairs of too low DNA quantity and quality for further genotyping. To estimate the accuracy of these newly designed Fluidigm genotyping assays, I compared genotypes of 17 cats called with both Sanger sequencing and Fluidigm. Genotyping error was 0.9%. To estimate the accuracy of the genotyping method optimized for hairs, I compared the genotypes generated from both tissue and single hair samples of selected individuals. Genotyping error was 1.6%. These low error rates allowed correct recognition of individuals and assessment of introgression levels. This optimized genotyping method thus allows monitoring introgression rate in natural populations based on non-invasive hair sampling.

In **chapter 3**, this optimized genotyping method was then applied to non-invasively and systematically collected hair samples of the cat population of the Swiss Jura, to assess its rate of introgression. Twenty one percent of the sampled wildcats were introgressed, based on the nuclear diagnostic markers. This corresponds to a migration rate from domestic cats to wildcats of 0.02 migrants per generation. In contrast, migration rate from wildcats into domestic cats was negligible, suggesting a directional introgression. Haphazard sampling of the same wildcat population, mostly via road kills, led to similar results. Hybridization was found to occur between wildcat male and domestic cat female as well as vice versa and, based on the occurrence of backcrosses, both female and male F1-hybrids seemed viable and fertile.

The hybridization patterns observed in chapter 3 were confirmed in **chapter 4**, where I estimated introgression rates in a large set of free-ranging wildcats of France, Switzerland and Germany. I found 53 hybrids (11%) out of 491 samples, corresponding to a migration rate from domestic cat to wildcat of 0.02 migrants per generation. Migration rate from wildcat into domestic cat was lower. Maternally inherited markers were more often introgressed than paternally inherited ones. Furthermore, hybrids seemed to concentrate at wildcat distribution edges. In addition, I found some evidence that the wildcat population of the Franco-Swiss Jura is possibly expanding. These results are all congruent with a selectively neutral model, where introgression could be seen as a mechanism of dispersal.

Although the main findings of this thesis suggests that introgression might simply be a byproduct of wildcat range expansion, it would be an overhasty conclusion to state that introgression is not a risk to wildcats, since many important aspects, e.g. demography, ecology and time, were not sufficiently considered so far. Based on the precautionary principle, introgression should still be considered as relevant to species conservation. A key conservation goal in respect to the potential threat of introgression should be the knowledge of the mechanisms leading to introgression. Thus, one of the main conservation measures should be to monitor introgression in wildcat populations over time.

Zusammenfassung

Introgression ist ein wichtiges Thema der Evolutionsbiologie. Sie wird definiert als Genfluss zwischen Taxa durch Hybridisierung über die erste Hybridengeneration hinaus. Eingekreuzte Gene eines nahe verwandten Taxons können als Rohmaterial für schnelle und adaptive evolutive Änderungen dienen. Introgression kann aber auch die Fitness der Hybriden vermindern (Auszuchtdepression), wenn die neu gemischten Merkmale schlecht an die Umgebung angepasst sind, oder wenn Genkomplexe, die sonst zusammen vererbt werden, durch die Kreuzung getrennt werden.

In der Artenschutzbiologie wird Introgression oft als Gefahr für die genetische Reinheit angesehen. Zudem gilt die Hybridisierung als besonders relevant, wenn sie durch den Menschen verursacht ist. Beides ist der Fall bei der Europäischen Wildkatze (*Felis silvestris silvestris*), die mit der Hauskatze (*Felis silvestris catus*) hybridisiert, möglicherweise seit über 2500 Jahren. Deshalb ist es für den Wildkatzenschutz wichtig, den Einfluss der Introgression abzuschätzen, indem freilebende Katzenpopulationen überwacht werden. Introgression zu erkennen ist allerdings schwierig. Bisher gab es weder morphologische noch genetische Methoden, um Introgression bei Wildkatzen verlässlich nachzuweisen.

Die vorliegende Doktorarbeit hatte zum Ziel, das Grundlagenwissen zu liefern, um den Einfluss der Introgression auf Wildkatzen abschätzen zu können. Dafür habe ich: 1) genetische Marker entwickelt, die Introgression erkennen können; 2) eine Genotypisiermethode für nicht-invasive Proben entwickelt, mit der eine Wildkatzenpopulation anhand von Haarproben überwacht werden kann; 3) die Introgressionsrate in der Wildkatzenpopulation aus dem Schweizer Jura geschätzt; 4) Hybridisierungsmuster bei Wildkatzen aus Frankreich, Deutschland und der Schweiz beschrieben.

Im **Kapitel 1** beschreibe ich die Entwicklung von Einzelnukleotid-Polymorphismen (Englisch: single nucleotide polymorphisms, SNP) Markern, welche erlauben, den individuellen Introgressionsgrad in Wildkatzen, Hauskatzen und deren Hybriden verlässlich zu erkennen. Zuerst definierte ich, welche Wild- und Hauskatzen als Referenztiere gelten, mittels Microsatellitenanalyse, Sequenzanalyse von Mitochondrium und Y-Chromosom und aufgrund morphologischer Kriterien. Zweitens sequenzierte ich einen ausgewählten kleinen Teil des Genoms von sechs Referenzwildkatzen und drei Referenzhauskatzen mit Hochdurchsatz-Sequenzierung. Der Vergleich der Sequenzen lieferte über 800‘000 polymorphe Nukleotide zwischen beiden Unterarten. Ich wählte dann 200 SNPs aus, an denen Wildkatzen und Hauskatzen unterschiedlich fixierte Allele hatten. Anschliessend sequenzierte ich die Genomregionen, in denen sich die SNPs befinden, in zusätzlichen zehn Wildkatzen und 13 Hauskatzen, um die Trennschärfe der 200 SNPs zu bestätigen. Schliesslich habe ich mit einem Bayesianischem Algorithmus untersucht, wie gut die 48 differenziertesten SNPs den Hybridisierungsgrad simulierter Hybriden bis zum zweiten Hybridisierungsgrad erkennen können. Mit diesem SNP-Set wurde die richtige Hybridenkategorie mit hoher Genauigkeit erkannt; 99.6% aller simulierten Individuen wurden richtig klassiert. Somit erlauben diese SNP-Marker, Introgressionsraten in natürlichen Populationen genau zu bestimmen.

Nicht-invasive Probenahme ist eine übliche und effiziente Art, um Proben schwer auffindbarer Populationen wie Wildkatzen zu erhalten. Allerdings ist die beschränkte Qualität und Menge nuklearer

DNS, die aus nicht-invasiven Proben gewonnen werden kann, eine Herausforderung für genaues Genotypisieren. **Kapitel 2** zeigt, wie ich eine SNP-Genotypisiermethode optimiert habe, um verlässliche Genotypen aus Einzelhaaren zu gewinnen. Ich habe einen 96.96 Fluidigm SNP genotyping array (SNP-Chip) entwickelt, basierend auf den in Kapitel 1 beschriebenen nuklearen diagnostischen SNP-Markern und publizierten mitochondrialen (mtDNS) SNPs. Der SNP-Chip enthielt die 75 nuklearen SNP-Marker, die Wildkatzen und Hauskatzen am besten unterschieden. Damit liess sich der individuelle Introgressionsgrad bestimmen. Zudem enthielt der Chip 11 nukleare Marker und vier mtDNA-Marker, um Individuen zu erkennen, sowie vier diagnostische mtDNA-Marker, um die mütterliche Linie zu verfolgen, und zwei diagnostische Y-Chromosom-Marker, um die väterliche Linie zu verfolgen und das Geschlecht zu bestimmen. Vor dem Genotypisieren wurde die aus Einzelhaaren gewonnene DNS-Menge mittels katzenspezifischer Echtzeit-PCR gemessen. Dieser Schritt erlaubt es, Haare auszuschliessen, die nicht von *Felis silvestris* stammen oder die zu wenig oder zu schlechte DNS für den folgenden Genotypisierungsschritt enthalten. Um die Zuverlässigkeit der neu entwickelten Fluidigm Genotypisierungsassays zu schätzen, verglich ich die Genotypen von 17 Katzen, die sowohl mit Sanger Sequenzierung als auch mit Fluidigm genotypisiert wurden. Die Genotypisierungsfehlerrate war 0.9%. Um die Zuverlässigkeit der für Einzelhaare optimierten Methode zu schätzen, verglich ich Genotypen von Gewebeproben und Einzelhaare von jeweils gleichen Individuen. Die Genotypisierungsfehlerrate war 1.6%. Diese tiefen Fehlerraten erlaubten, Individuen und deren Introgressionsgrad zuverlässig zu bestimmen. Damit ermöglicht die optimierte Genotypisierungsmethode, die Introgressionsrate in freilebenden Populationen anhand nicht-invasiver Haarproben zu überwachen.

In **Kapitel 3** wurde diese Methode schliesslich auf nicht-invasiv und systematisch erhobene Haarproben der Katzenpopulation aus dem Schweizer Jura angewandt, um deren Introgressionsrate zu bestimmen. Einundzwanzig Prozent der beprobten Wildkatzen zeigten aufgrund der nuklearen diagnostischen Marker Introgression. Dies entspricht einer Migrationsrate von Haus- zu Wildkatzen von 0.02 Migranten pro Generation. Die Migrationsrate von Wild- zu Hauskatzen hingegen war vernachlässigbar, was auf eine gerichtete Introgression hinweist. Eine willkürliche Beprobung der gleichen Wildkatzenpopulation, mehrheitlich bestehend aus Verkehrsopfern, führte zu ähnlichen Ergebnissen. Hybridisierung kam sowohl zwischen Wildkater und Hauskatzen als zwischen Wildkatzen und Hauskater vor und sowohl weibliche als auch männliche F1-Hybriden schienen aufgrund der beobachteten Rückkreuzungen lebensfähig und fruchtbar.

Die in Kapitel 3 festgestellten Hybridisierungsmuster wurden in **Kapitel 4** bestätigt, wo ich die Introgressionsrate in einem grösseren Datenset freilebender Wildkatzen aus Frankreich, Deutschland und der Schweiz geschätzt habe. Es wurden 53 Hybriden (11%) aus 491 Proben gefunden, was einer Migrationsrate von Haus- zu Wildkatzen von 0.02 Migranten per Generation entspricht. Die Migrationsrate von Wild- zu Hauskatzen war geringer. Mütterlich vererbte Marker waren häufiger eingekreuzt als Marker auf der väterlichen Linie. Zudem schienen die Hybriden sich am Rand der Wildkatzenverbreitung zu konzentrieren. Es ergaben sich auch Hinweise, dass sich die Wildkatzenpopulation im Französisch-Schweizerischem Jura ausbreitet. Diese Ergebnisse würden mit

einem selektiv neutralen Modell übereinstimmen, wonach Introgression ein Verbreitungsmechanismus sein könnte.

Obwohl die Hauptergebnisse dieser Arbeit andeuten, dass Introgression womöglich schlicht eine Nebenerscheinung einer sich ausbreitenden Wildkatzenpopulation ist, wäre es verfrüht daraus zu schliessen, dass Introgression kein Risiko für Wildkatzen darstellt, da viele Kernaspekte wie Demographie, Ökologie und Zeit noch nicht genügend berücksichtigt wurden. Gemäss dem Vorsorgeprinzip sollte Introgression weiterhin als artenschutzrelevant betrachtet werden. Es sollte ein Hauptziel des Artenschutzes vor der Introgressionsgefahr sein, die Mechanismen der Introgression zu kennen. Eine wichtige Schutzmassnahme wäre deshalb, die Introgression in den Wildkatzenpopulationen längerfristig zu überwachen.

General Introduction

About introgression and cats

Beatrice Nussberger

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Introgression: chance or risk?

Introgression is defined as the flow of genes between taxa through hybridization beyond the first generation of hybrids. Hybridization is a common process, occurring in at least 10% of animal species and 25% of plant species, especially in young species, having reproduction barriers that are still weak (Mallet 2005). Introgression can be an important evolutionary force (Barton 2001; Grant et al. 2004; Mallet 2005; Arnold 2006; Mallet 2007). Hybrids can be fitter than their parents in new, extreme or perturbed habitats. For example, hybrids of Darwin's finches (*Geospiza fortis x G. scandens*), survived better than their parents after a sudden change of ecological conditions (Grant & Grant 2008). Introgression increases genetic variation and as such allows a more rapid response to selection than if genetic variation is only generated by mutation. Introgression can also counteract negative effects of inbreeding depression (Grant et al. 2003) or can even lead to adaptive evolution (Lewontin & Birch 1966; Dasmahapatra et al. 2012). Direct hybrid speciation, although considered to be relatively rare, can occur, if first generation hybrids (F1) have a mate preference for their hybrid conspecifics, as shown in butterflies *Heliconius sp.* (Mavarez et al. 2006). Hybrids have therefore the potential to contribute positively to species evolution.

On the other hand, introgression can lead to outbreeding depression, that is, reduced fitness in hybrids. Outbreeding depression can be extrinsic, by introducing maladapted traits leading to a loss of adaptation, or intrinsic, by disrupting co-adapted gene complexes leading to genetic incompatibility (Keller et al. 2000; Lancaster et al. 2007; Huff et al. 2011). Based on Darwin's theory of evolution (Darwin 1859), hybrids are likely to be maladapted and thus under negative selection. But selection against unfit hybrids may not be strong enough to prevent large scale introgression, especially if a rare species hybridizes with a more abundant species (Allendorf et al. 2001). As a consequence, genetic swamping can occur, even if hybrids are less viable (Epifanio & Philipp 2000). Genetic swamping might yet result in the genetic extinction of a taxon (Rhymer & Simberloff 1996).

From a conservation perspective, it is further important to distinguish between natural and anthropogenic hybridization (Allendorf et al. 2001). Natural hybrids should be eligible for protection, whereas anthropogenic hybrids have *a priori* a lower conservation value. Gene flow from domesticated species into wild relatives is particularly relevant in plants, when transgenes invade natural populations or when domestic crops are implicated in the extinction of their wild forms (Ellstrand et al. 1999; Haygood et al. 2003). But anthropogenic hybridization also concerns animals (dogs and wolves: Randi & Lucchini 2002; farmed and wild salmons: McGinnity et al. 2003; cattle and bisons: Halbert & Derr 2007; pigs and wild boars: Goedbloed et al. 2013). An extreme case is the one of the American black ducks (*Anas rubripes*), which are at risk to go extinct through genetic assimilation with mallard ducks (*Anas platyrhynchos*). The genetic integrity of black ducks diminished drastically over the 20th century, due to human mediated habitat alteration and game-farm mallards releases (Mank et al. 2004). In fact, translocations of organisms and habitat modifications through humans increase introgression occurrences (Rhymer & Simberloff 1996; Simberloff 1996). However, introgression of domesticated genes is not necessarily negative. It may introduce genetic variation in

the wild population upon which selection can act, facilitating rapid evolutionary changes, as shown in the Soay sheep (Feulner et al. 2013).

Difficulties to detect introgression

Studying introgression, not to mention defining sensible conservation goals about introgression, is very difficult, as long as one does not even know how to recognize hybrids and their offspring. Hybrids and especially further introgressed individuals often look like one of their parents, which makes it impossible to assess the hybridization level based on morphology only (Barbour et al. 2007; Krüger et al. 2009; Seiler et al. 2009; Ostberg et al. 2011). Genetic methods can help discover cryptic hybrids (Schwartz et al. 2004). However, detecting hybrids beyond the first generation, that is, introgressed individuals, is more difficult, even with genetics.

Genetic methods commonly used to detect hybridization are based on mitochondrial DNA (mtDNA) and/or autosomal microsatellites. These genetic markers have several drawbacks. The mtDNA only reflects the evolution of the mitochondrial organelles of the maternal line. Hence, only female biased hybridization can be recognized. Mitochondrial organelles have often a different evolutionary history than reflected by the nuclear DNA of the organism carrying them (Roca et al. 2005; Bachtrog et al. 2006; Petit & Excoffier 2009; Hedrick 2010; Hailer et al. 2012; Zielinski et al. 2013). Incomplete lineage sorting, i.e. retention of ancient polymorphisms, or mitochondrial capture can lead to shared mitochondrial haplotypes between taxa. The general drawback by applying microsatellites for detecting introgression beyond first generation hybrids is that it takes many of them to obtain reliable conclusions (Vähä & Primmer 2006). This is in particular true when microsatellites are polymorphic and not diagnostic, because they share alleles in both taxa (Figure 1). In addition, many reference animals are needed to reliably determine the allele frequencies based on which the parental populations can be differentiated. Furthermore, studies using different microsatellite markers and in different numbers result in different estimates of hybridization rates and are not comparable.

Currently, the advances of genomic techniques allow better performing approaches to detect introgression (Twyford & Ennos 2012). For example, several studies used next-generation sequencing to discover diagnostic markers in hybridizing fishes (Hohenlohe et al. 2011; Amish et al. 2012). The new molecular techniques offer the advantage that they allow a broader screening of the genome of more individuals in less time and costs than in the recent past.

Study system: European Wildcats hybridize with domestic cats

European wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis silvestris catus*) belong to the same polytypic species (Driscoll et al. 2007). Hybridization between these two subspecies is known to occur throughout Europe. Several studies mention hybrids in Scotland (Beaumont et al. 2001), Italy

(Randi et al. 2001), Hungary (Pierpaoli et al. 2003; Lecis et al. 2006), Portugal and Spain (Oliveira et al. 2008), Switzerland (Stoeckle 2008), Germany (Hertwig et al. 2009) and France (O'Brien et al. 2009).

European wildcats (*Felis silvestris silvestris*) and African wildcats (*Felis silvestris libyca*), i.e. the assumed ancestor of the domestic cat, evolved into two distinct subspecies for about 230'000 years (Driscoll et al. 2007). A low level of natural gene flow between both subspecies, i.e., introgression, probably still occurs, especially in areas where both subspecies are sympatric. The divergence time being very recent, the genetic distance between both taxa is expected to be relatively low and mating compatibility remains high (Mallet 2005). In addition, European wildcats face gene flow from the domestic cat, *Felis silvestris catus,* genetically close to his ancestor *Felis silvestris libyca*. Domestication of *Felis silvestris libyca* started at least 9'000 years ago (Vigne et al. 2004). Humans introduced domestic cats into the distribution range of wildcats since Roman times (Faure & Kitchener 2009). Human mediated hybridization is thus possible since then. At least since the 19th century, the wildcat populations decreased drastically due to persecution as well as loss and fragmentation of habitat (Schauenberg 1970). At this time, domestic cats were increasingly bred in Europe. In the middle of the 20th century, wildcats got protected in several countries, e.g. 1976 in France, 1952 in Germany, 1962 in Switzerland, and their populations could recover since then (Nussberger et al. 2007). In parallel, also domestic cats most probably increased. For example, between 1995 and 2010, the Swiss domestic cat population was estimated to have increased from 1.2 to 1.5 million (Verband für Heimtiernahrung, www.vhn.ch). The increase in density of both cat populations could have favored encounters and thus hybridization between both subspecies.

Even if behavioral barriers do exist (Hubbard et al. 1992) and mating between wildcats and domestic cats may not be common, introgression can still occur. Introgression with domestic cats is commonly handled as a threat to wildcats (Driscoll & Nowell 2010), since introgression can lead to genetic swamping and even genetic extinction (Rhymer & Simberloff 1996). However, introgression levels in wildcats could not be assessed so far. Morphologic methods fail to reliably distinguish hybrids and their backcrossed offspring from pure wildcats (Figure 2; Nussberger & Weber 2007; Krüger et al. 2009; Devillard et al. submitted 2013) and the microsatellite marker sets used so far had only limited power to detect hybrids beyond the first generation (Say et al. 2012).

This thesis

To objectively judge positive or negative effects of introgression in natural populations, one needs first to be able to recognize introgressed individuals and subsequently to assess the rate of introgression in these populations. This knowledge is crucial to investigate the consequences of introgression on the fitness of wildcat populations. Inferring introgression patterns, such as sex-bias in hybridization, can further help defining conservation measures.

In the present thesis, I aimed to provide this basic knowledge by developing genetic markers able to disclose introgression (chapter 1), establishing a genotyping method for non-invasive samples that allow monitoring a wildcat population based on hair samples (chapter 2), assessing the introgression rate in the wildcat population of the Jura region (chapter 3) and finally describing hybridization patterns in wildcats from France, Germany and Switzerland (chapter 4).

Chapter 1 describes the development of genetic markers allowing distinguishing between wildcats, domestic cats and also their introgressed progeny. These are biallelic markers, called SNPs (single nucleotide polymorphisms), for which wildcats and domestic cats have different allele frequencies (differentiation index $F_{ST} > 0.8$). Due to the strong differentiation between wildcats and domestic cats at these nucleotides, these markers are diagnostic, i.e. they allow detecting wildcats, domestic cats and hybrids, even beyond the first generation. To discover these diagnostic markers, I used reduced representation libraries, high-throughput sequencing and Sanger sequencing.

Once I found the genetic markers useful to detect admixture, I had to establish a genotyping method that allows high throughput genotyping of low quality samples. This new genotyping protocol, presented in **Chapter 2**, includes a DNA quantification step with real-time PCR and uses a SNP chip allowing simultaneous genotyping of 96 SNP in 96 samples. The SNP chip relies on very short assays (<150 base pairs), which makes it suitable for non-invasively collected samples, like hair samples or faeces, which are known to contain low amounts of DNA and highly fragmented DNA (Vigilant 1999). This step was important since it is difficult to obtain high quality samples from an elusive species like the wildcat. One successful way of sampling wildcats is based on lure stick traps, which allow collecting hairs from the animals rubbing their fur against the lure stick (Hupe & Simon 2007).

Chapter 3 shows the introgression rate observed in a natural wildcat population, based on cat hair samples systematically collected throughout the Swiss Jura (Weber et al. 2008). Introgression rate was also assessed based on ad libitum collected samples. This led to similar results. The migration rate between domestic cats and wildcats was of 0.02 migrants per generation. The gene flow was asymmetric and mostly directed from domestic cats to wildcats.

Finally, in **Chapter 4**, I explored hybridization patterns in wildcats from France, Germany and Switzerland. Expanding the wildcat sampling over the Swiss borders did not change the introgression rate, which was again estimated to be 0.02 domestic migrants per generation. Introgression was asymmetric: maternally inherited mtDNA was more introgressed than paternally inherited Y-chromosome. Hybrids seem to be mostly at local edges of wildcat distribution and close to dense human settlements. In addition, there is molecular evidence for wildcat population growth. These observed patterns might be explained by an expanding wildcat population.

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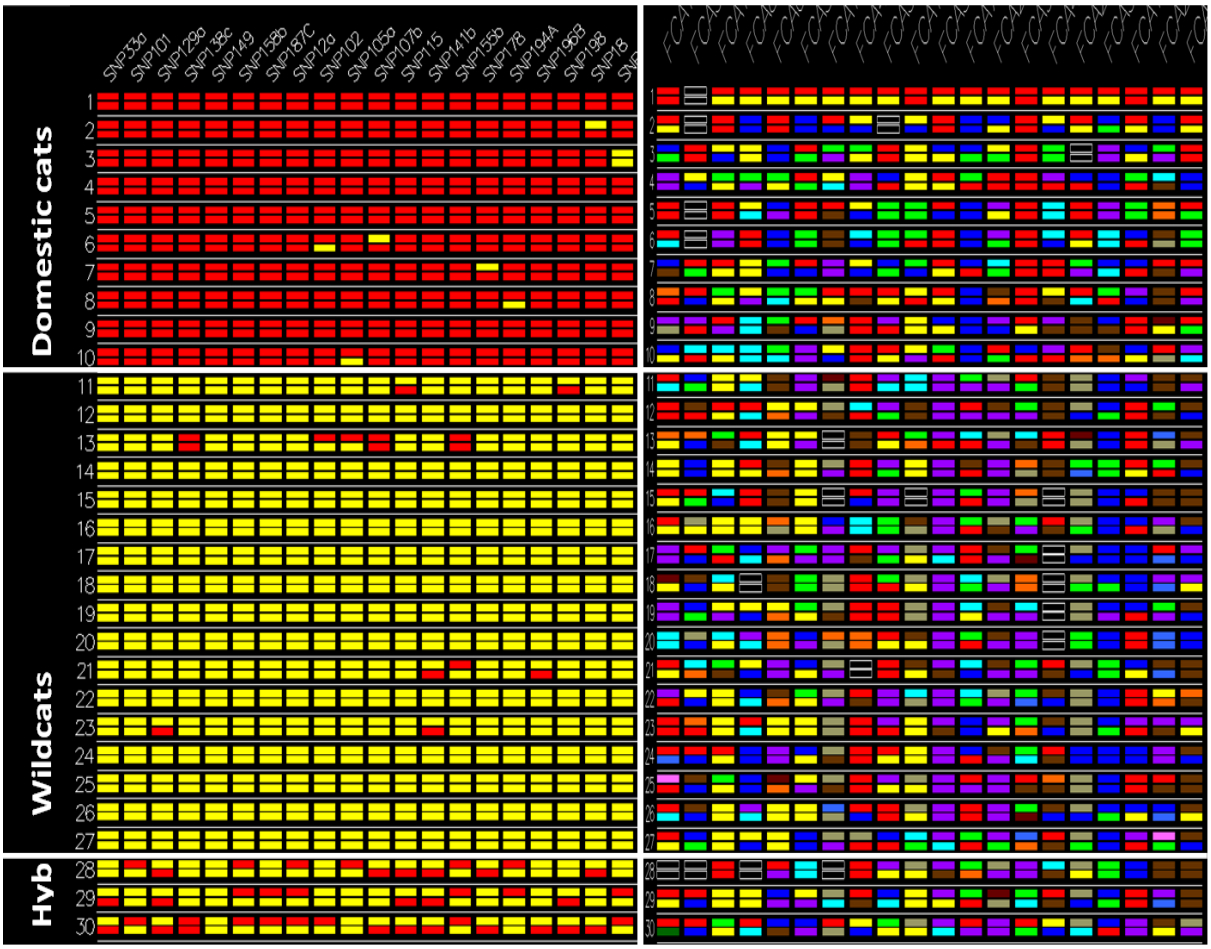

Figure 1: Diagnostic single nucleotide polymorphism (SNP) markers developed in this thesis (left) allow recognizing second generation wildcat hybrids, whereas microsatellite markers usually do not (right). Every column represents a genetic marker and every row corresponds to one individual cat with two alleles for each marker. Different colors represent different alleles. These SNP markers have only two alleles and together are diagnostic. One allele (red) is much more frequent in domestic cats whereas the other allele (yellow) is more frequent in wildcats. Microsatellites are highly polymorphic and same alleles can occur in both wildcats and domestic cats. Individuals 1-10 are domestic cats; individuals 11-27 are wildcats; individuals 28-30 are hybrids (Hyb), 28-29 are backcrosses into wildcats and 30 is a first generation hybrid.

Figure 2: Hybrids (left) and wildcats (right) have similar phenotypes. © FIWI, Berne

Chapter 1

Development of SNP markers identifying European wildcats, domestic cats, and their admixed progeny

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Abstract

Introgression can be an important evolutionary force but it can also lead to species extinction and as such is a crucial issue for species conservation. However, introgression is difficult to detect, morphologically as well as genetically. Hybridization with domestic cats (*Felis silvestris catus*) is a major concern for the conservation of European wildcats (*Felis silvestris silvestris*). The available morphologic and genetic markers for the two *Felis* subspecies are not sufficient to reliably detect hybrids beyond first generation. Here we present a single nucleotide polymorphism (SNP) based approach that allows the identification of introgressed individuals. Using high-throughput sequencing of reduced representation libraries we developed a diagnostic marker set containing 48 SNPs (Fst>0.8) which allows the identification of wildcats, domestic cats, their hybrids and backcrosses. This allows assessing introgression rate in natural wildcat populations and is key for a better understanding of hybridization processes.

Introduction

Introgression is difficult to detect, yet it is an important issue in evolutionary biology and conservation. Introgression, the flow of genes between taxa through hybridization beyond the first generation of hybrids (F1), can be an important evolutionary force (Grant *et al.*, 2004; Seehausen, 2004; Grant, Grant, 2009) and can also lead to species extinction (Rhymer, Simberloff, 1996). Introgression is especially a conservation concern when it is anthropogenic (Allendorf *et al.*, 2001). This is the case in the crosses between European wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis s. catus*). To assess the threat caused by hybridization, we need to quantify the introgression rate in potentially threatened populations. Therefore, it is crucial to overcome the difficulties in detecting not only F1, but also introgressed individuals, which are the decisive hybrids from a conservation perspective (Allendorf *et al.*, 2001).

Introgression is difficult to detect for several reasons. First, morphological criteria are frequently not useful, since hybrids beyond the F1 generation are morphologically often indistinguishable from the parental species (Barbour *et al.*, 2007; Krüger *et al.*, 2009; Seiler *et al.*, 2009; Ostberg *et al.*, 2011). In wildcats for example, even the distinction between parentals of both hybridizing taxa based on morphology alone has been questioned (Daniels *et al.*, 1998; Nussberger, Weber, 2007). Second, the genetic identification is challenging because introgressed individuals share a large part of their genome with one of the parental species. For instance, a first generation backcross shares on average 75% of its genes with the parental species in which it has backcrossed. Consequently, many genetic markers are required to detect the presence of genes from the other parental species, especially when markers are highly polymorphic and not diagnostic, e.g. microsatellites (Vähä, Primmer, 2006). For backcross detection, single nucleotide polymorphism (SNP) markers appear promising. SNPs are mostly biallelic (Lai, 2001) and they cannot be more than tetrallelic (A, C, G or T). Due to the low number of alleles and the low degree of homoplasy, SNP markers are more likely

to be diagnostic than highly polymorphic markers. Therefore, SNP markers are particularly useful for detecting introgressed hybrids. For example, diagnostic SNP markers have been used to detect introgression in hybridizing fish taxa (Finger *et al.*, 2009; Simmons *et al.*, 2009; Hohenlohe *et al.*, 2011; Amish *et al.*, 2012).

Here we report a SNP-based approach that allows the identification of introgressed individuals, and we illustrate it with data from European wildcats. European wildcats are known to hybridize and to have fertile offspring with domestic cats (Beaumont *et al.*, 2001; Randi *et al.*, 2001; Pierpaoli *et al.*, 2003; Lecis *et al.*, 2006; Oliveira *et al.*, 2008b; Hertwig *et al.*, 2009; O'Brien, 2009). Hybridization with domestic cats is considered one of the major threats to the wildcat in many European countries (Driscoll, Nowell, 2010). There is a need to better recognize and understand the processes and extent of introgression in order to develop appropriate conservation measures. However, the microsatellite marker sets commonly used to distinguish between wildcats and domestic cats have limited power to distinguish introgressed individuals, that is, hybrids beyond the F1 generation (Oliveira *et al.*, 2008a; Oliveira *et al.*, 2008b; Hertwig *et al.*, 2009; Say *et al.*, 2012). In Hertwig et al. (2009), 3.5% of simulated F2 and 47% of simulated backcrosses were misinterpreted as parentals. In Oliveira et al. (2008b), 12% of simulated F2 and 20% of simulated backcrosses were erroneously attributed to parentals. Clearly, a set of more powerful markers is needed to assess the level of introgression in natural wildcat populations and the degree of threat to wildcats. Here, we aimed to obtain a set of diagnostic SNP markers for identifying wildcats, domestic cats, as well as their hybrids and backcrosses, by identifying single nucleotide polymorphisms in the genome where wild- and domestic cats present markedly different allele frequencies, using high-throughput sequencing of reduced representation libraries and Sanger sequencing.

Materials and Methods

Methodological strategy

In a first step we defined what we considered domestic cats and wildcats (*reference samples*), using morphology and genetic data of a total of 45 potential domestic cats and 33 potential wildcats. Subsequently we sequenced a small part of the genome (*reduced representation library*) of six wildcats and three domestic cats. The comparison between the sequences of wildcats and domestic cats revealed SNPs between both subspecies. We then selected 200 SNPs at which our wildcat and domestic cat samples showed differently fixed alleles (*SNP selection*). To validate their diagnostic value, these SNPs were genotyped in an additional ten wildcats and 13 domestic cats by Sanger sequencing (*SNP validation*). Finally, we tested if our markers can assess the hybrid status of simulated individuals with known hybrid status (*SNP power assessment*).

Reference samples

Domestic cat samples (blood, gonads, hairs) were provided by Swiss veterinary offices and private cat owners (n=35). We assumed that all these cats were domestic, since they lived in close proximity with humans and were tame. Moreover, most of the domestic cat samples came from regions where wildcats are absent. Eleven of these domestic cats were purebred. In addition to these domestic cats, gamekeepers provided samples from ten stray cats with domestic phenotype, from regions where wildcats occur (Supporting Information Table 1).

Blood or tissue samples from potential wildcats of the Swiss Jura region were provided by the Centre for Fish and Wildlife Health in Berne, Switzerland, by gamekeepers and by the Natural History Museums of Basel, Berne, La Chaux-de-Fonds, Lausanne, Neuchatel and Olten (n=33, SI Table 1). We defined the reference wildcats according to both genetic and morphologic criteria. We followed the genetic identification method suggested by Driscoll et al. (2011), with a modified set of markers. We genotyped all potential wildcats at 24 autosomal microsatellites (Menotti-Raymond *et al.*, 1999) and one Y linked microsatellite (Luo *et al.*, 2007). In addition, we sequenced 2698bp of the mitochondrial DNA genes ND5 and ND6 (Driscoll *et al.*, 2007) and 376bp of SRY and 366bp of SMCY on the Y chromosome (Pecon-Slattery *et al.*, 2004; King *et al.*, 2007). A complete list of markers with their primer sequences is provided in SI Table 2. For comparison, we further generated the same genetic data for 30 domestic cats from various breeds. Population substructure among all wildcats and these 30 domestic cats was identified using STRUCTURE (Pritchard *et al.*, 2000). Mitochondrial DNA and Y-chromosomal haplotypes sequence data were compared with published haplotype sequences (Driscoll *et al.*, 2007) in GENEIOUS Pro 4.8.5 Software (Drummond AJ, 2009) to ascertain wildcat specificity. We only considered samples as reference wildcats if the animals carried wildcat mtDNA and Y haplotypes and if the proportion of autosomal wildcat ancestry (q value) was ≥ 0.95 according to STRUCTURE. Furthermore, when pictures from the sampled wildcats were available, we checked if the genetic results corresponded to the classic morphologic criteria: permanent dorsal line stopping at base of tail, blunt tail tip, distinct tail bands, four stripes on nape, two stripes on shoulder, blurry broken stripes on flanks, rhinarium with upper black margin and gularis with white areola (Ragni, Possenti, 1996; Kitchener *et al.*, 2005).

Reduced representation library RRL

To achieve high enough coverage for SNP detection with a given amount of sequencing effort, we chose to sequence only a small portion (2%) of the genome. To this end we constructed reduced representation libraries (RRL) by digesting genomic DNA and size selecting fragments (Van Tassell *et al.*, 2008). Genomic DNA was extracted from six reference wildcat samples and three domestic cat samples (Biosprint 96 DNA Blood kit, Qiagen). The six wildcats used for implementing the RRL were selected to have different geographical origins throughout the Swiss Jura region, thus reducing the likelihood of having related individuals in the sample. To construct RRLs, we digested 25 μg of genomic DNA with 250 Units of HaeIII (New England Biolabs). We separated the digested genomic DNA on a Spreadex EL1200 Wide Mini S-2x4 gel (Elchrom Scientific) in a SEA 2000 electrophoresis

chamber at 55°C, 120 Volt, in 1x TAE running buffer (Elchrom Scientific), during three hours. We excised fragments between 587 bp and 622 bp. To extract DNA fragments, we placed gel pieces in a dialysis membrane (Carl Roth, 1785.1 Dialysierschlauch Visking, Cellulose) filled with 1x TAE buffer and closed it with plastic clips (Carl Roth, H277.1 ZelluTrans/Roth Verschlussklammer). The membrane packages were placed in an electrophoresis chamber (SEA 2000) at 55°C, 120 Volt, in 1x TAE running buffer, during approximately 45 minutes. We purified the eluate using the MinElute PCR Purification Kit (Qiagen) according to the manufacture's protocol. We prepared the sequencing library and individually barcoded our samples following the instructions of the SOLiD™ 4 System Library Preparation Guide (Applied Biosystems, 2010). The sequencing library was only amplified with eight PCR cycles to minimize over-amplification. After DNA quantification with qPCR (SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit, Applied Biosystems), each sample was diluted to 500pM. We submitted pooled libraries to the Functional Genomics Center Zurich (FGCZ) who performed paired-end (50/35) sequencing on SOLiD 4 (Applied Biosystems).

SNP selection

Raw sequence reads from SOLiD 4 platform were mapped to the cat genome assembly version FelCat4 (Pontius *et al.*, 2007) using the default settings in Bioscope version 1.3.1 (Life Technologies, Carlsbad, CA, USA). SNPs were called using DiBayes (Life Technologies, Carlsbad, CA, USA) with high and medium stringency settings. To be able to compare genotypes of all individuals at a given SNP site, Samtools version 0.1.12a (Li *et al.*, 2009) was used in cases where no call was made by DiBayes to check whether the SNP site was not sequenced or homozygous for the reference allele.

From these SNPs, we selected potentially diagnostic SNPs based on three criteria. First, SNPs had to be sequenced to at least ten times coverage in all samples. Second, SNPs had to be fixed for a different allele in wildcats and domestic cats, meaning that the polymorphism at the SNP was only found between and not within subspecies. Third, we only selected markers which were on different chromosomes or at least 10kb from one another, since unlinked markers are best for hybrid detection. We verified fixed SNPs visually with the Integrative Genomics Viewer (Robinson *et al.*, 2011).

SNP validation

As we only obtained genomic data of nine cats in our initial SNP detection, we verified the allelic state of 200 potentially diagnostic SNPs in up to 23 additional cats by PCR and Sanger sequencing, thereby generating a total of 32 reference cat genotypes (16 wildcats and 16 domestic cats). For each locus, we therefore designed PCR primer pairs (Primer 3, Rozen, Skaletsky, 2000) to obtain PCR products of 200 to 799 bp encompassing these potentially diagnostic SNPs (SI Table 3). PCR conditions were 30 cycles with annealing at 59°C (57°C for SNP082). We sequenced these products using Big Dye Terminate v3.1 chemistry on a 3730 DNA Analyzer (Applied Biosystems). Subsequently we analysed sequence data with Sequencing Analysis 5.1. (Applied Biosystems) and edited them in GENEIOUS. The number of individuals to be sequenced per locus was determined by calculating the F_{ST}-values between wild- and domestic cats with the individuals already analysed.

When F_{ST} was <0.7 after sequencing eight or 16 individuals, we did not further analyse this locus. F_{ST}-values were calculated as the difference between the expected heterozygosity in wild- and domestic cats taken together and the mean of the expected heterozygosity in wild- and domestic cats separately, divided by the expected heterozygosity in wild- and domestic cats taken together (Conner, Hartl, 2004).

SNP power assessment

We wanted to assess the power of the 48 SNP markers with highest F_{ST}-values (> 0.8) in determining the correct hybrid status of simulated hybrids. To simulate hybrid genotypes, we needed genotypes for parental wildcats and domestic cats. To identify parental cats we first genotyped these 48 SNP markers in 42 additional cats, which had not been used to classify the markers based on F_{ST}-values. Using new genotypes avoids the "high-grading" bias in assessing power of marker sets described by Anderson (2010). The 42 additional individuals comprised 18 domestic cats, ten stray cats, seven reference wildcats and seven potential wildcats with unclear status due to a contradiction between mtDNA or Y marker and autosomal microsatellites. We then used the program NEWHYBRIDS Version 1.1 Beta (Anderson, Thompson, 2002) to assess the posterior probability of belonging to the following six categories for each of these 42 samples: parental wildcats (W), parental domestic cats (D), first generation hybrids (F1), second generation hybrids (F2, i.e. F1 x F1), backcrosses into wildcats (BxW, i.e. F1 x W), backcrosses into domestic cats (BxD, i.e. F1 x D). We used the default parameters of the program NEWHYBRIDS and did not include any other individuals in this analysis than these 42 samples. All samples which had ≥ 0.95 posterior probability of belonging to the parental categories D or W were used as parental samples to simulate F1, F2 and backcrossed hybrids.

We created the genotypes of hybrids F1, F2 and backcrosses (BxD and BxW) by sampling without replacement from amongst the alleles in the parental samples, using R 2.9.2 (RDevelopementCoreTeam, 2009). Sampling the parental alleles without replacement avoids the problem of simulating lots of hybrid individuals that all carry a copy of the same allele in the parental sample. However, it limits the number of hybrids that can be generated. We simulated as many hybrids and backcrosses as we had parental alleles to distribute. For example, with nine parental wildcats, we had 18 alleles to create 18 F1 (in combination with nine domestic cats, resp. 18 domestic alleles) or 12 BxW (in combination with six domestic alleles needed for six F1). We analysed the simulated hybrids in NEWHYBRIDS, each hybrid category separately. In each NEWHYBRIDS run, we included the genotypes of the defined pure 16 wildcats and 16 domestic cats that were used in the RRL and SNP validation steps as known parentals, using the z and s option of NEWHYBRIDS. We repeated the simulation and analysis steps 200 times for each hybrid category. We defined individuals as correctly assigned by NEWHYBRIDS when their true category was the category with the highest scaled likelihood. Scaled likelihoods are the posterior probabilities to belong to a certain hybrid category, under a model where a priori every one of the hybrid categories is equally likely. We calculated the percentage of correctly assigned individuals (accuracy) and the mean scaled likelihoods (posterior probabilities) of all simulated individuals per category.

Furthermore, to explore the extent to which hybridization beyond the second hybrid generation is detectable with our method, we simulated using four additional categories of hybrids: crosses between a backcross into wildcat and a parental wildcat (BxW x W) and between backcross into wildcat and F1 (BxW x F1) and the same for domestic cats (BxD x D, BxD x F1). We analysed simulated individuals of all ten categories separately with NEWHYBRIDS, allowing for ten genotype frequency classes (2 parentals, F1, F2, BxW, BxD, BxWxW, BxWxF1, BxDxD, BxDxF1).

Results

Reference samples

We identified 24 reference wildcats based on microsatellites, mtDNA and Y markers. We had pictures of 19 of these cats. The phenotype of all these cats fulfilled the usual wildcat criteria. Nine potential wildcats were possibly of admixed ancestry and thus were not considered as reference wildcats: two potential wildcats (WK050, WK054) showed evidence of possible introgression at the autosomal microsatellites ($q < 0.95$ in STRUCTURE), and seven potential wildcats were of wildcat ancestry at the nuclear markers with $q \geq 0.95$ but mtDNA or Y markers were of the domestic cat type. All 43 domestic cats and stray cats which were analysed with microsatellite markers, mtDNA and/or Y markers were confirmed as domestic cats (SI Table 1).

RRL

The sequencing of the reduced representation libraries of six wildcats and three domestic cats yielded 597,139,577 sequenced beads. About 48% of these beads (285,234,154), representing a total of 11.5 gigabases, could be mapped to the reference Cat Genome.

SNP selection

At 654 out of 876,690 called SNP positions, all RRL samples were sequenced at least ten times and were fixed for alternate alleles in domestic and wild cats. However, when these fixed SNPs were verified within the Integrative Genomics Viewer, several of these SNP positions contained additional alleles, although at low coverage and mostly with a low Phred quality score (<20). As a consequence we selected by eye the 200 SNPs displaying the lowest number of reads with an alternate allele (SI Table 3).

SNP validation

Table 1 shows the positions of 187 potentially diagnostic SNPs on the domestic cat reference genome (FelCat4 december 2008, Pontius *et al.*, 2007) and gives the corresponding allele frequencies for wildcats and domestic cats. Differences in allele frequencies are graphically shown in Figure 1. We excluded 13 markers (6.5%) because of primer mismatch, indel-allele or multiple product amplification (e.g. primer binding region in a repeated element, SI Table 3). Overall, F_{ST}-

values for the SNPs ranged from zero to one. Fifty SNPs had an F_{ST}-value of >0.8 between wildcats and domestic cats, including seven SNPs with F_{ST} =1 (Table 1, F_{ST}).

SNP power assessment

Based on 48 nuclear SNP markers with F_{ST}-values > 0.8, NEWHYBRIDS assigned 41 of the 42 additionally genotyped cats with >0.95 posterior probability to one of six possible categories. All 18 domestic cats and ten stray cats were classified as parental domestic cats. Three reference wildcats (WK026, WK041, WK045) and one wildcat with domestic Y marker (WK024) were classified as backcrosses into wildcat. Three reference wildcats (WK017, WK035, WK049) and six wildcats with domestic mtDNA marker (WK020, WK022, WK027, WK036, WK055, WK077) were classified as parental wildcats. One reference wildcat (WK145) was classified as parental wildcat, but with a posterior probability of only 0.77 and was therefore excluded for hybrid simulation. Thus we had 28 parental domestic cats and nine parental wildcats to simulate hybrid genotypes.

NEWHYBRIDS assigned 99.6% of simulated individuals to the correct hybrid category with > 0.50 posterior probability when using the 48 SNPs with highest F_{ST}-values (Table 2). 97.3% of the simulated individuals were assigned with > 0.95 posterior probability to their true category. The mean posterior probabilities to belong to the true category was >0.98 for all simulated categories (Table 3).

Using only 32 of the SNPs with highest F_{ST}-values slightly lowered the mean posterior probabilities of belonging to either hybrid category, but, overall, still 98.6% of all individuals were correctly categorized. With 24 markers, the accuracy was still 97.7% (data not shown).

In the NEWHYBRIDS analysis of third generation hybrids, still 86.5% of simulated individuals were correctly assigned and the posterior probabilities for the ten simulated categories were around 0.8 (Tables 2 and 3). Eight percent of the parental domestic cats and 18% of the parental wildcats were erroneously categorized as third generation hybrids. However, in all hybrid categories, less than 1% of all simulated hybrids were assigned to the parental groups. Thus, while not all parental are correctly identified as such, hybrids are recognized correctly with high probability, although not always assigned to the correct hybrid category.

Discussion

First and second generation hybrids are reliably recognized with our set of SNP markers. We were able to identify the hybrid category of 97.3% of all simulated individuals with a posterior probability of >0.95, using 48 markers with highest F_{ST}-values (F_{ST} > 0.8). Even when including third generation hybrids, our marker set still allowed the correct identification of 86.5.% of the simulated individuals. Thus, our new approach to detect SNP markers does work well in the case of the wildcats, domestic cats and their hybrids. Our approach consisted in sequencing a similar fraction of the genome of reference animals from both parental taxa, selecting SNPs diagnostic in these reference animals and

verifying these SNPs in additional individuals. This marker development protocol will also be useful to find diagnostic SNPs in other hybridizing species.

Choosing the right reference samples to develop diagnostic markers is crucial, yet challenging. First, reference samples should not contain any hybrids, as this will reduce the chances of correctly identifying diagnostic markers. Second, for the method to be broadly applicable, reference samples should be representative of the genetic diversity in the parental populations. Every wildcat found in Europe today is a potential hybrid, since domestic cats are thought to have spread in the area of the European Wildcat (*Felis silvestris silvestris*) since Roman times (Faure, Kitchener, 2009). Ideally, we therefore would have developed the markers using wildcat samples from before Roman times, i.e. from more than 2'500 years ago. But ancient DNA is of low quality and quantity (Hofreiter *et al.*, 2001) and reduced representation libraries require DNA of high quality and quantity. Thus, we instead analysed modern samples with 24 autosomal markers, mtDNA sequences and Y markers. Samples without any sign of hybridization in all these markers were defined as reference wildcats. These reference wildcats formed a genetically distinct group relative to domestic cats. We minimized the probability of having introgressed individuals in our domestic cat reference sample by using mainly domestic cats from regions far from the habitat of wildcats in Switzerland (Jura region). To ensure adequate representation of genetic diversity in our reference samples we used domestic cats from different breeds and regions and we included wildcats from across their range in Switzerland. We cannot tell at present whether these markers are also applicable to wildcats beyond the Swiss borders. But we expect the differentiation between wildcats and domestic cats to be much higher than the differentiation between wildcat populations within Europe. Therefore, we hypothesize our marker set is also applicable to samples from outside Switzerland. Preliminary results of samples from France, Italy, Germany, Hungary and Bulgaria, which we genotyped with a 96x96 SNP genotyping chip (data not shown), support this hypothesis. Still, we would encourage researchers to test the markers in a larger set of known reference samples from other countries. Further, our markers are tested only for the subspecies *Felis silvestris silvestris* and *catus*. Their applicability to other *Felis silvestris* subspecies remains to be investigated.

Our simulations for the SNP power assessment are subject to potential bias. As some introgression between wildcats and domestic cats is expected, only clearly differentiated individuals were used as parental animals for the simulations of hybrid categories. As a consequence, the samples used for the simulations may be enriched with individuals more differentiated than average. This can lead to an overestimation of the SNP power for hybrid identification, because the detection of a hybrid is easier the more differentiated the two parental animals are. However, we expect this bias to be small here, given the strong differentiation of the SNPs between both subspecies.

High-throughput sequencing allows detecting a high number of markers at once and thus seems to be the method of choice for future marker development (Twyford, Ennos, 2012). In addition, it is often sufficient to sequence only a small part of the genome (Davey *et al.*, 2011), as we did here with RRL. Recently, a similar approach using RAD tags was described for SNP discovery in trouts (Hohenlohe *et al.*, 2011). A slightly different approach of detecting diagnostic markers was chosen by Karlsson et

al. (2011), who found genetic differences between farmed and wild Atlantic salmon based on a 7K SNP-chip. All these high-throughput sequencing approaches offer the advantage of generating markers that cover a broad range of the genome.

Different genetic questions need different genetic markers (Sunnucks, 2000; Freeland, 2005). Reliably recognizing hybrids beyond F1 has proven difficult with highly polymorphic microsatellite markers in several species (Fur seal: Kingston, Gwilliam, 2007; Wildcats: Oliveira *et al.*, 2008a; Hertwig *et al.*, 2009; Say *et al.*, 2012; Florida bog frogs: Austin *et al.*, 2011). In theory as few as four to five fully diagnostic markers would be sufficient to identify recent backcrosses (Boecklen, Howard, 1997). In our data, 24 almost diagnostic SNP markers were sufficient to correctly categorize 97.7% of all simulated hybrids, using a threshold for posterior probability of > 0.5. However, with highly polymorphic, non diagnostic microsatellites, it takes about 48 markers to recognize backcrossed individuals with a posterior probability of > 0.5 (Vähä, Primmer, 2006). Most of the studies of hybridization in wildcats used between nine and 27 microsatellite markers, with allelic richness between seven and 43 (Beaumont *et al.*, 2001; Randi *et al.*, 2001; Pierpaoli *et al.*, 2003; Lecis *et al.*, 2006; Oliveira *et al.*, 2008b; O'Brien, 2009). Markers with high allelic richness, like microsatellites, are well suited to recognize genetic population structure (Guichoux *et al.*, 2011). However, high allelic richness in combination with homoplasy reduces the diagnostic power of markers for hybrid recognition, since there are more possibilities of allele sharing between two hybridizing taxa. Therefore, highly polymorphic markers developed for detecting genetic population structure are not the best markers to identify introgression. It is worth developing diagnostic markers with the explicit intent to detect introgression. The drawback of the diagnostic markers is that they should not be used for other genetic analyses such as genetic differentiation measures or paternity tests. On the other hand, the RRL approach we used for the diagnostic marker development generates enough high-throughput sequencing data to allow the development of other markers for other purposes as well.

SNPs are powerful markers to detect introgression. Their power resides in the highly differentiated allele frequencies between hybridizing taxa. Although high discriminatory power can also be reached with microsatellites (Burgarella *et al.*, 2009), SNP markers present several advantages over microsatellite markers. SNPs are mostly biallelic. In our screening of 200 regions around a potentially diagnostic SNP, we found over 360 SNPs. Only two of them were triallelic (in sequence of SNP091 and SNP136) and none were tetrallelic. At biallelic SNPs, a diploid has only three options per locus: homozygous for either of the alleles, or heterozygous. This makes hybrid detection straightforward, at least in fixed SNPs. Heterozygosity at all SNP positions indicate a F1 hybrid and an individual having a proportion of 75% of the alleles from one parental is most probably a backcross into that parental group. SNPs have also several technical advantages over microsatellites. Results obtained in different laboratories are compatible without a need to calibrate them. SNP genotyping assays are easier to multiplex than microsatellites, because they do not rely on the detection of fragment length. Finally, SNP genotyping assays can be designed to be very short, e.g. using PCR products shorter than 100 bp, because only a single base position has to be determined. This allows working with

highly fragmented DNA and low DNA quantities, as is found in faeces, hair or ancient samples (Morin, McCarthy, 2007).

In the near future, we aim to genotype non-invasively collected hair samples from free ranging wildcats to assess the introgression rate of domestic cats into different European wildcat populations. Depending on levels of introgression, management plans for species conservation can then be developed (Allendorf *et al.*, 2001). Overall, our set of novel SNP markers allows the reliable assessment of introgression levels in natural populations and thus will help improve our understanding of the process of hybridization and introgression.

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B.N., P.W., L.F.K. designed research; B.N. performed research; M.P.G. contributed new analytical tools; C.G. ran the simulations.

The authors declare no conflict of interest.

Data Accessibility

The following data are available on Dryad `doi:10.5061/dryad.270b7`

mtDNA sequences: Fasta File containing mtDNA sequences of wild- and domestic cats

MicrosatelliteData: Excel File containing microsatellite allele length results for all individuals

MorphologyData: Excel File containing description of diagnostic morphology criteria for all individuals

RefSamplesSequences: Folder containing the raw sequences (subfolder SNPsequences) and the annotated consensus sequences (subfolder SNPconsensusSequences) for each of the 200 diagnostic SNPs

SNPgenotypingData: Excel File containing SNP genotyping results for all individuals

SimulationFiles: Folder containing R script for hybrid simulations (SimsNewHybrids.R), input files (SelectionSimulOnlyRefs040912.txt, SelectionSimulOnlyTestInd040912.txt) and file that holds the definitions of ten of the genotype frequency classes possible after three generations of mating between two species (TwoGensGtypFreq10.txt)

Y_data: Folder containing the raw sequences from SRY sequencing (SNPseqSRY) and the SMCY microsatellite allele length for male individuals (SMCYdata)

Supporting information

Additional supporting information in *Molecular Ecology Resources (2013)* ***13****, 447-460*:

SItable1_Individuals: description of individuals (origin, morphology, Y, mtDNA, microsatellites)

SItable2_Markers: list of primers used to define reference samples

SItable3_SNPprimers: list of primers used for SNP genotyping by sequencing

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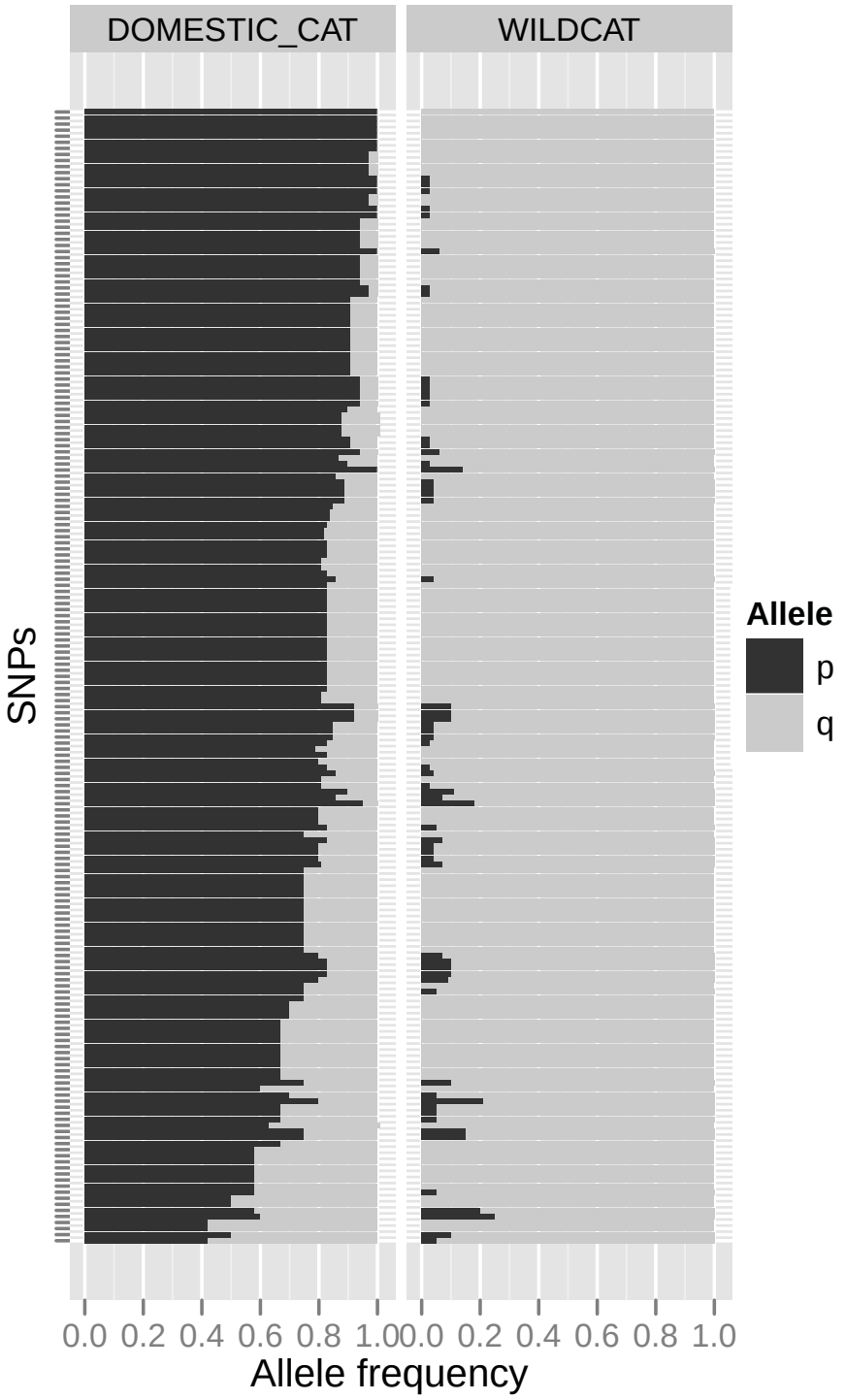

Figure 1: Allele frequencies for both alleles p and q in domestic cats and wildcats at 187 SNP markers. Every horizontal bar represents one of the 187 SNP positions. The SNPs are ordered along the vertical axis according to decreasing F_{ST}-values between wildcats and domestic cats.

Table 1. List of 187 SNP markers to detect introgression in domestic cats (D) and wildcats (W). Chromo_Position indicates the position of the SNP on the cat reference Genome, FelCat4 (Pontius *et al.*, 2007, version Dec. 2008). F_{ST} is a measure of genetic differentiation between D and W. p and q are the two alleles found at the SNP position. nD and nW indicate the number of D and W successfully genotyped at the SNP position. p in D and q in W represent the frequencies of the alleles in the two subspecies. Nr gives the rank of the SNP after sorting by F_{ST}, SNP Nr is the identification number.

Nr	SNP Nr	Chromo_ Position	F_{ST}	p	q	nD	p in D	q in D	nW	p in W	q in W
1	33	C1_133254300	1	T	G	16	1	0	16	0	1
2	101	B4_143164026	1	C	T	16	1	0	16	0	1
3	129	B4_96741303	1	G	A	16	1	0	16	0	1
4	138	C2_142773339	1	G	A	16	1	0	16	0	1
5	149	A3_157140228	1	A	C	16	1	0	16	0	1
6	158	B3_37642991	1	C	T	16	1	0	16	0	1
7	187	D3_49022779	1	C	G	16	1	0	16	0	1
8	12	A3_90799249	0.94	G	T	16	0.97	0.03	16	0	1
9	102	C2_142858667	0.94	C	T	16	0.97	0.03	16	0	1
10	105	D2_106505320	0.94	C	T	16	0.97	0.03	16	0	1
11	107	E2_51498305	0.94	C	A	16	0.97	0.03	16	0	1
12	115	A2_63544109	0.94	G	A	16	1	0	16	0.03	0.97
13	141	E1_47366937	0.94	G	A	16	1	0	16	0.03	0.97
14	155	B2_129152112	0.94	A	C	16	1	0	16	0.03	0.97
15	178	C1_189621758	0.94	G	A	16	0.97	0.03	16	0	1
16	194	E1_125241814	0.94	C	T	16	0.97	0.03	16	0	1
17	196	E2_50523470	0.94	T	A	16	1	0	16	0.03	0.97
18	198	E3_13634364	0.94	T	C	16	1	0	16	0.03	0.97
19	18	B1_58403280	0.88	C	A	16	0.94	0.06	16	0	1
20	32	C1_118678562	0.88	C	G	16	0.94	0.06	16	0	1
21	62	D2_88876341	0.88	G	T	16	0.94	0.06	16	0	1
22	93	B3_28741053	0.88	C	T	16	0.94	0.06	16	0	1
23	109	F2_65362892	0.88	G	A	16	0.94	0.06	16	0	1
24	133	C1_163375181	0.88	G	A	16	1	0	16	0.06	0.94
25	139	D4_75458793	0.88	T	C	16	0.94	0.06	16	0	1
26	148	A2_120724549	0.88	G	A	16	0.94	0.06	16	0	1
27	162	B3_99865718	0.88	G	A	16	0.94	0.06	16	0	1
28	192	D4_51926783	0.88	G	A	16	0.94	0.06	16	0	1
29	193	D4_52053226	0.88	C	T	16	0.94	0.06	16	0	1
30	184	D2_2202956	0.88	C	T	16	0.97	0.03	16	0.03	0.97
31	195	E2_33320051	0.88	A	G	16	0.97	0.03	16	0.03	0.97
32	14	B1_123418311	0.83	A	G	16	0.91	0.09	16	0	1
33	28	B4_143439104	0.83	G	A	16	0.91	0.09	16	0	1
34	41	D4_37998587	0.83	T	C	16	0.91	0.09	16	0	1
35	48	A3_51056949	0.83	C	T	16	0.91	0.09	16	0	1
36	57	D1_98155760	0.83	T	C	16	0.91	0.09	16	0	1
37	58	D1_126067118	0.83	G	A	16	0.91	0.09	16	0	1
38	60	D1_128802001	0.83	A	T	16	0.91	0.09	16	0	1
39	65	D3_76217054	0.83	G	T	16	0.91	0.09	16	0	1
40	88	F2_9296568	0.83	A	G	16	0.91	0.09	16	0	1
41	146	A1_214220499	0.83	C	T	16	0.91	0.09	16	0	1
42	176	C1_112821482	0.83	T	C	16	0.91	0.09	16	0	1
43	189	D3_73181465	0.83	C	T	16	0.91	0.09	16	0	1
44	190	D3_88773687	0.83	C	G	16	0.91	0.09	16	0	1
45	20	B2_132559340	0.82	A	G	16	0.94	0.06	16	0.03	0.97
46	26	B3_75494376	0.82	G	C	16	0.94	0.06	16	0.03	0.97
47	30	B4_45476816	0.82	A	G	16	0.94	0.06	16	0.03	0.97
48	159	B3_39998169	0.82	A	C	16	0.94	0.06	16	0.03	0.97
49	166	B3_147841323	0.82	G	A	16	0.94	0.06	16	0.03	0.97
50	50	C1_223335334	0.82	G	T	15	0.90	0.10	15	0	1

51	98	E1_47901546	0.78	G	A	16	0.88	0.13	15	0	1
52	1	A1_214461789	0.78	G	C	16	0.88	0.13	16	0	1
53	64	D3_70959423	0.78	A	G	16	0.88	0.13	16	0	1
54	126	B3_102961557	0.78	G	C	16	0.88	0.13	16	0	1
55	67	E2_64389936	0.77	G	T	16	0.91	0.09	16	0.03	0.97
56	152	B1_168327330	0.77	G	A	16	0.91	0.09	16	0.03	0.97
57	127	B3_132539085	0.77	C	T	16	0.94	0.06	16	0.06	0.94
58	106	D4_36844519	0.76	A	C	15	0.87	0.13	16	0	1
59	21	B2_38455848	0.76	C	T	15	0.90	0.10	16	0.03	0.97
60	66	E2_28834826	0.76	G	A	10	1	0	14	0.14	0.86
61	177	C1_177165193	0.74	C	G	14	0.86	0.14	16	0	1
62	7	A2_36537402	0.74	G	T	14	0.89	0.11	14	0.04	0.96
63	38	D2_16797246	0.74	A	G	14	0.89	0.11	14	0.04	0.96
64	114	A2_62528310	0.74	G	A	14	0.89	0.11	14	0.04	0.96
65	90	A1_80251090	0.73	G	A	14	0.89	0.11	13	0.04	0.96
66	136	C2_10551765	0.73	G	A	13	0.85	0.15	13	0	1
67	27	B4_106165338	0.73	C	T	16	0.84	0.16	16	0	1
68	143	F2_29878116	0.73	C	T	16	0.84	0.16	16	0	1
69	96	D4_61706901	0.71	C	T	15	0.83	0.17	16	0	1
70	153	B2_11210402	0.70	G	A	14	0.82	0.18	14	0	1
71	36	D1_9247995	0.69	C	G	14	0.82	0.18	15	0	1
72	151	B1_57974383	0.69	C	T	9	0.83	0.17	13	0	1
73	17	B1_24516687	0.69	G	A	6	0.83	0.17	9	0	1
74	95	B4_106085849	0.69	T	G	6	0.83	0.17	9	0	1
75	89	F2_29604098	0.69	C	T	16	0.81	0.19	15	0	1
76	173	B4_122774768	0.68	T	C	16	0.81	0.19	16	0	1
77	6	A2_22115264	0.68	A	C	9	0.83	0.17	14	0	1
78	84	D4_103411241	0.68	A	G	14	0.86	0.14	14	0.04	0.96
79	10	A3_150434747	0.68	A	G	6	0.83	0.17	10	0	1
80	19	B2_11748866	0.68	G	A	6	0.83	0.17	10	0	1
81	37	D2_15700028	0.68	T	C	6	0.83	0.17	10	0	1
82	45	F1_24323263	0.68	T	C	6	0.83	0.17	10	0	1
83	56	D1_72733259	0.68	A	C	6	0.83	0.17	10	0	1
84	69	F1_31149992	0.68	C	T	6	0.83	0.17	10	0	1
85	72	F2_64410099	0.68	A	G	6	0.83	0.17	10	0	1
86	76	B3_3763474	0.68	G	A	6	0.83	0.17	10	0	1
87	80	C2_134622594	0.68	G	A	6	0.83	0.17	10	0	1
88	83	D4_60140710	0.68	G	A	6	0.83	0.17	10	0	1
89	100	A2_154972126	0.68	T	C	6	0.83	0.17	10	0	1
90	113	A1_267376697	0.68	A	G	6	0.83	0.17	10	0	1
91	160	B3_67119952	0.68	T	C	6	0.83	0.17	10	0	1
92	163	B3_104962724	0.68	C	G	6	0.83	0.17	10	0	1
93	167	B4_2696116	0.68	C	T	6	0.83	0.17	10	0	1
94	175	C1_88089878	0.68	T	C	6	0.83	0.17	10	0	1
95	199	F2_4630496	0.68	A	G	6	0.83	0.17	10	0	1
96	200	F2_21635256	0.68	A	G	6	0.83	0.17	10	0	1
97	63	D2_111465892	0.67	A	G	13	0.81	0.19	14	0	1
98	181	D1_999750	0.67	C	T	13	0.81	0.19	14	0	1
99	51	C2_64959967	0.66	G	A	6	0.92	0.08	10	0.10	0.90
100	134	C1_188295633	0.66	G	T	6	0.92	0.08	10	0.10	0.90
101	174	C1_6047515	0.66	T	C	6	0.92	0.08	10	0.10	0.90
102	168	B4_2713634	0.65	G	A	10	0.85	0.15	14	0.04	0.96
103	170	B4_44289069	0.65	T	A	10	0.85	0.15	14	0.04	0.96
104	171	B4_44832398	0.65	C	A	10	0.85	0.15	14	0.04	0.96
105	164	B3_130995527	0.65	A	C	15	0.83	0.17	16	0.03	0.97
106	8	A2_6906598	0.65	C	G	14	0.79	0.21	14	0	1
107	86	F1_85116491	0.64	T	C	6	0.83	0.17	13	0	1
108	15	B1_191096484	0.64	T	A	10	0.80	0.20	14	0	1
109	49	B4_147077847	0.64	C	T	12	0.83	0.17	16	0.03	0.97
110	82	D3_124203045	0.64	G	A	7	0.86	0.14	13	0.04	0.96
111	71	F2_45763245	0.63	G	T	8	0.81	0.19	14	0	1

112	111	A1_222959361	0.63	G	A	16	0.81	0.19	16	0.03	0.97
113	16	B1_20092839	0.62	A	G	10	0.90	0.10	14	0.11	0.89
114	74	A1_239785943	0.62	C	T	14	0.86	0.14	14	0.07	0.93
115	61	D1_129618021	0.61	C	T	10	0.95	0.05	14	0.18	0.82
116	52	C2_74163720	0.59	A	G	5	0.80	0.20	10	0	1
117	142	F1_20032493	0.59	G	A	5	0.80	0.20	10	0	1
118	197	E2_66138174	0.59	T	G	5	0.80	0.20	10	0	1
119	42	E1_72880071	0.59	G	A	6	0.83	0.17	10	0.05	0.95
120	188	D3_60909701	0.59	G	C	6	0.75	0.25	7	0	1
121	157	B3_6909289	0.58	C	G	12	0.83	0.17	14	0.07	0.93
122	122	B2_84861747	0.58	A	G	10	0.80	0.20	13	0.04	0.96
123	35	C2_45117916	0.57	C	T	10	0.80	0.20	14	0.04	0.96
124	180	C2_137811507	0.57	G	T	10	0.80	0.20	14	0.04	0.96
125	40	D3_32104510	0.57	T	C	16	0.81	0.19	15	0.07	0.93
126	54	C2_140733169	0.56	G	A	10	0.75	0.25	14	0	1
127	4	A1_9371605	0.54	A	G	6	0.75	0.25	10	0	1
128	11	A3_169913387	0.54	T	A	6	0.75	0.25	10	0	1
129	13	A3_93714149	0.54	T	C	6	0.75	0.25	10	0	1
130	22	B2_67536455	0.54	G	A	6	0.75	0.25	10	0	1
131	24	B3_57147258	0.54	A	G	6	0.75	0.25	10	0	1
132	55	D1_68082963	0.54	T	C	6	0.75	0.25	10	0	1
133	117	A3_28148083	0.54	C	T	6	0.75	0.25	10	0	1
134	147	A2_42383186	0.54	G	A	6	0.75	0.25	10	0	1
135	154	B2_71247052	0.54	T	C	6	0.75	0.25	10	0	1
136	165	B3_135866504	0.54	G	T	6	0.75	0.25	10	0	1
137	179	C2_68465481	0.54	G	A	6	0.75	0.25	10	0	1
138	191	D4_10426918	0.54	T	C	6	0.75	0.25	10	0	1
139	99	F1_26460636	0.53	C	T	4	0.75	0.25	7	0	1
140	183	D1_109313008	0.52	C	A	10	0.80	0.20	14	0.07	0.93
141	2	A1_269159716	0.51	G	A	6	0.83	0.17	10	0.10	0.90
142	29	B4_15403984	0.51	G	A	6	0.83	0.17	10	0.10	0.90
143	59	D1_128044982	0.51	C	G	6	0.83	0.17	10	0.10	0.90
144	156	B2_134892585	0.50	C	A	15	0.80	0.20	16	0.09	0.91
145	73	E3_33733408	0.50	G	A	4	0.75	0.25	8	0	1
146	47	F2_7927040	0.45	G	C	6	0.75	0.25	10	0.05	0.95
147	145	A1_151348480	0.44	C	A	4	0.75	0.25	10	0	1
148	104	C2_158469278	0.44	C	G	5	0.70	0.30	9	0	1
149	130	B4_111855682	0.41	A	G	5	0.70	0.30	10	0	1
150	132	C1_82808777	0.41	A	G	5	0.70	0.30	10	0	1
151	3	A1_274277184	0.41	A	G	6	0.67	0.33	10	0	1
152	46	F2_3749961	0.41	C	A	6	0.67	0.33	10	0	1
153	79	C1_30344863	0.41	A	G	6	0.67	0.33	10	0	1
154	81	D1_11065896	0.41	A	G	6	0.67	0.33	10	0	1
155	92	B1_202073444	0.41	A	G	6	0.67	0.33	10	0	1
156	103	C2_151794647	0.41	C	T	6	0.67	0.33	10	0	1
157	124	B3_77335049	0.41	A	G	6	0.67	0.33	10	0	1
158	128	B3_148360238	0.41	C	T	6	0.67	0.33	10	0	1
159	137	C2_11113978	0.41	G	A	6	0.67	0.33	10	0	1
160	182	D1_88915301	0.41	T	C	6	0.67	0.33	10	0	1
161	140	D4_104246955	0.38	C	T	6	0.75	0.25	10	0.10	0.90
162	116	A2_200475325	0.36	C	T	5	0.60	0.40	7	0	1
163	43	E2_23114722	0.34	A	G	5	0.70	0.30	10	0.05	0.95
164	44	E3_12301230	0.34	A	G	10	0.80	0.20	14	0.21	0.79
165	25	B3_73330050	0.33	T	C	6	0.67	0.33	10	0.05	0.95
166	131	C1_34406063	0.33	A	G	6	0.67	0.33	10	0.05	0.95
167	135	C1_207927310	0.33	G	A	6	0.67	0.33	10	0.05	0.95
168	87	F2_2358597	0.33	G	A	8	0.63	0.38	14	0	1
169	34	C1_50675581	0.33	C	A	6	0.75	0.25	10	0.15	0.85
170	112	A1_247553760	0.33	C	T	6	0.75	0.25	10	0.15	0.85
171	9	A3_143339672	0.31	G	T	3	0.67	0.33	7	0	1
172	5	A2_176836753	0.29	T	C	6	0.58	0.42	10	0	1

173	31	B4_80349376	0.29	A	C	6	0.58	0.42	10	0	1
174	75	A2_130163447	0.29	C	T	6	0.58	0.42	10	0	1
175	85	E3_12162520	0.29	T	A	6	0.58	0.42	10	0	1
176	91	A3_100831036	0.29	G	A	6	0.58	0.42	10	0	1
177	121	A3_126916218	0.29	C	T	6	0.58	0.42	10	0	1
178	161	B3_71735716	0.29	C	G	6	0.58	0.42	10	0	1
179	108	F2_18305725	0.23	A	C	6	0.58	0.42	10	0.05	0.95
180	68	E2_64946728	0.18	A	G	6	0.50	0.50	10	0	1
181	77	B3_140493835	0.18	G	C	6	0.50	0.50	10	0	1
182	185	D2_9756017	0.11	C	T	6	0.58	0.42	10	0.20	0.80
183	110	F2_68402465	0.08	A	G	5	0.60	0.40	10	0.25	0.75
184	78	B4_52463921	0.08	A	G	6	0.42	0.58	10	0	1
185	118	A3_31797110	0.08	A	T	6	0.42	0.58	10	0	1
186	119	A3_73505900	0.05	T	C	5	0.50	0.50	10	0.10	0.90
187	125	B3_78472523	0.05	T	G	6	0.42	0.58	10	0.05	0.95

Table 2. Power assessment with NEWHYBRIDS using 48 SNP markers with highest F_{ST} values (F_{ST}> 0.8). Assignments to each hybrid category from a number n of simulated genotypes from the following categories: parental wildcat (W), parental domestic cat (D), F1, F1xF1 (F2), backcross into wildcat (BxW), backcross into domestic cat (BxD) and beyond second generation also BxD x D, BxD x F1, BxW x W and BxW x F1. Number of correct assignments are highlighted in bold. Accuracy gives the percentage of correct assignments. Each individual was assigned to the category for which the posterior probability was highest based on a NEWHYBRIDS analysis.

	True	Category assessed with the highest probability											
	Category	D	W	F1	F2	BxD	BxW	BxDxD	BxWxW	BxDxF1	BxWxF1	n	Accuracy
until second generation	**D**	**1793**	0	0	0	7	0	-	-	-	-	1800	99.6
	W	0	**1793**	0	0	0	7	-	-	-	-	1800	99.6
	F1	0	0	**3589**	10	1	0	-	-	-	-	3600	99.7
	F2	0	0	0	**3592**	5	3	-	-	-	-	3600	99.8
	BxD	0	0	0	12	**2388**	0	-	-	-	-	2400	99.5
	BxW	0	0	0	22	0	**2378**	-	-	-	-	2400	99.1
until third generation (ten categories)	**D**	**1654**	0	0	0	0	0	146	0	0	0	1800	91.9
	W	0	**1474**	0	0	0	0	0	326	0	0	1800	81.9
	F1	0	0	**3588**	5	0	0	0	0	4	3	3600	99.7
	F2	0	0	18	**2664**	4	0	0	0	445	469	3600	74.0
	BxD	0	0	0	0	**2090**	0	107	0	203	0	2400	87.1
	BxW	0	0	1	0	0	**2068**	0	82	0	249	2400	86.2
	BxDxD	20	0	0	0	250	0	**2128**	0	2	0	2400	88.7
	BxWxW	2	0	0	0	0	282	0	**2110**	0	6	2400	87.9
	BxDxF1	0	0	0	304	104	0	0	0	**1992**	0	2400	83.0
	BxWxF1	0	0	0	300	0	62	0	0	2	**2036**	2400	84.8

Table 3. Mean posterior probabilities and 99% confidence intervals of belonging to a defined hybrid category for a number n of simulated genotypes. Categories are: parental wildcat (W), parental domestic cat (D), F1, F1xF1 (F2), backcross into wildcat (BxW), backcross into domestic cat (BxD) and for the simulations of hybrids beyond second generation BxD x D, BxD x F1, BxW x W and BxW x F1. Values for the correct categories are highlighted in bold.

	True Categ.	Mean posterior probabilities and 99% confidence intervals										n
		D	**W**	**F1**	**F2**	**BxD**	**BxW**	**BxDxD**	**BxWxW**	**BxDxF1**	**BxWxF1**	
until second generation	**D**	**0.994 ± 0.003**	0 ± 0	0 ± 0	0 ± 0	0.006 ± 0.003	0 ± 0	-	-	-	-	1800
	W	0 ± 0	**0.994 ± 0.003**	0 ± 0	0 ± 0	0 ± 0	0.006 ± 0.003	-	-	-	-	1800
	F1	0 ± 0	0 ± 0	**0.993 ± 0.002**	0.006 ± 0.002	0.001 ± 0.001	0 ± 0	-	-	-	-	3600
	F2	0 ± 0	0 ± 0	0 ± 0	**0.995 ± 0.002**	0.003 ± 0.002	0.002 ± 0.001	-	-	-	-	3600
	BxD	0 ± 0	0 ± 0	0 ± 0	0.012 ± 0.003	**0.988 ± 0.003**	0 ± 0	-	-	-	-	2400
	BxW	0 ± 0	0 ± 0	0 ± 0	0.016 ± 0.005	0 ± 0	**0.984 ± 0.005**	-	-	-	-	2400
until third generation (ten categories)	**D**	**0.879 ± 0.013**	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.121 ± 0.013	0 ± 0	0 ± 0	0 ± 0	1800
	W	0 ± 0	**0.789 ± 0.019**	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.21 ± 0.019	0 ± 0	0 ± 0	1800
	F1	0 ± 0	0 ± 0	**0.991 ± 0.003**	0.004 ± 0.001	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.003 ± 0.001	0.002 ± 0.001	3600
	F2	0 ± 0	0 ± 0	0.005 ± 0.003	**0.642 ± 0.011**	0.001 ± 0.001	0 ± 0	0 ± 0	0 ± 0	0.171 ± 0.01	0.18 ± 0.01	3600
	BxD	0 ± 0	0 ± 0	0 ± 0	0.002 ± 0	**0.789 ± 0.013**	0 ± 0	0.074 ± 0.009	0 ± 0	0.136 ± 0.011	0 ± 0	2400
	BxW	0 ± 0	0 ± 0	0 ± 0.001	0.001 ± 0	0 ± 0	**0.798 ± 0.013**	0 ± 0	0.056 ± 0.008	0 ± 0	0.145 ± 0.012	2400
	BxDxD	0.017 ± 0.005	0 ± 0	0 ± 0	0 ± 0	0.147 ± 0.012	0 ± 0	**0.833 ± 0.013**	0 ± 0	0.003 ± 0.001	0 ± 0	2400
	BxWxW	0 ± 0	0.002 ± 0.002	0 ± 0	0 ± 0	0 ± 0	0.151 ± 0.013	0 ± 0	**0.843 ± 0.014**	0 ± 0	0.004 ± 0.002	2400
	BxDxF1	0 ± 0	0 ± 0	0 ± 0	0.191 ± 0.012	0.062 ± 0.009	0 ± 0	0.001 ± 0.001	0 ± 0	**0.742 ± 0.013**	0.004 ± 0.001	2400
	BxWxF1	0 ± 0	0 ± 0	0 ± 0	0.19 ± 0.012	0 ± 0	0.041 ± 0.007	0 ± 0	0 ± 0	0.005 ± 0.001	**0.765 ± 0.013**	2400

Chapter 2

A SNP chip to detect introgression in wildcats allows accurate genotyping of single hairs

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Abstract

Genotyping non-invasively collected samples is challenging. Nevertheless, genetic monitoring of elusive species like the European Wildcat (*Felis silvestris silvestris*) mainly relies on such samples. Wildcats are likely threatened through introgression with domestic cats (*Felis silvestris catus*). To determine introgression based on single cat hairs, we developed a 96.96 Fluidigm single nucleotide polymorphism genotyping array (SNP chip). To estimate the accuracy of this method, we compared genotypes of 17 cats called with both Sanger sequencing and Fluidigm. Considering Sanger sequencing genotypes as a reference, the genotyping error rate with Fluidigm was 0.9%. We subsequently compared 16 hair samples to tissue samples of the same individual. Using the tissue samples as reference, the genotyping error rate in hair samples was 1.6%. This low error rate allowed reliable recognition of individuals and correct assessment of introgression levels. Thus, the here presented genotyping method is suitable for non-invasively collected samples. It will help conservationists to monitor the introgression rate in wildcat populations based on non-invasive hair sampling and subsequently to conduct effective conservation measures.

Introduction

The limited quality and quantity of nuclear DNA extracted from non-invasively collected samples, like single hairs (Vigilant 1999; Bengtsson et al. 2011), is a challenge for accurate genotyping (Gagneux et al. 1997; Goossens et al. 1998). The currently applied methods of pooling hairs from the same individual to increase DNA quantity and multiple genotyping to assess error rates (Taberlet et al. 1997; Goossens et al. 1998) are usually not applicable to hairs collected on hair traps. In fact, pooling of hairs from lure stick traps can lead to erroneous hybrid genotypes, when hairs belong to different individuals, and the low amount of DNA extracted from single hairs does often not allow multiple genotyping. Nevertheless, conservation and population genetic studies often rely on non-invasively collected samples, because it is an efficient way to sample elusive species (Valière et al. 2003; Schwartz et al. 2004; Henry & Russello 2011; Heurich et al. 2012; Barbosa et al. 2013). For instance, non-invasive hair sampling using lure stick traps has been put forward as a useful way to survey European wildcats (Felis silvestris silvestris; Kéry et al. 2011; Steyer et al. 2013).

Introgression with domestic cats (*Felis silvestris catus*) is thought to be a threat to European wildcat (Daniels et al. 2001; Oliveira et al. 2008; Randi 2008; Driscoll & Nowell 2010), which could lead to its genetic extinction (Rhymer & Simberloff 1996). Thus, it is crucial to monitor and better understand the process of introgression in wildcat populations. However, the assays used so far to monitor wildcats based on hairs do not recognize introgression (Hertwig et al. 2009; Say et al. 2012) and the assays developed to recognize introgression are not suitable for hair samples (Nussberger et al. 2013).

In the present study, we provide a method that tackles these challenges to assess the introgression rates in wildcats based on non-invasive hair sampling using lure stick traps. We investigated I)

whether the newly designed single nucleotide polymorphism (SNP) chip is reliably reflecting genotypes generated with Sanger sequencing and II) whether this chip yields reliable genotypes even in samples of low DNA quality and quantity, such as single hairs. Moreover, we present a new set of SNP genotyping assays for high throughput genotyping of European wildcats and domestic cats, allowing to recognize individuals and to assess individual introgression levels from hair samples.

Materials and methods

Cat samples were provided by the Centre for Fish and Wildlife Health in Berne, gamekeepers and private collections. Blood and tissue samples were extracted using the DNeasy Blood & Tissue kit (QIAGEN), following the manufacturer's protocol. Hair samples were extracted with the Sample-to-SNP-kit (Applied Biosystems) using the following modified protocol. We checked every hair under the microscope for the presence of a root, placed each hair root singly into a 0.2ml PCR tube, added 9μl Lysis Solution and placed the tube in a thermocycler at 75°C for 10 min and 95°C for 4 min. Finally, we added 9μl Stabilization Solution. Hair samples were plucked hairs, stored dry at room temperature for 15 to 53 month prior to DNA extraction.

We quantified the cat specific DNA amount available for genotyping in 16 singly extracted hairs (four single hairs from four individuals) using quantitative real-time-PCR on a StepOnePlus instrument (Applied Biosystems). PCR containing 2μl DNA, 10μl FastStart Universal SYBR Green Master (ROX) 2x (Roche Applied Science), 6.64μl molecular grade water, 0.16μl BSA, 0.6μl forward and 0.6μl reverse cat specific cMyc primer of 10μM (F: ACGCACAACGTCTTGGAAC; R: TGGCCTTTTTAAGGATCACC). Initial incubation was set to 10 min at 95°C, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. Melt curve stage was 95°C for 15 s, 60°C for 1 min (step and hold +0.3°C) and 95°C for 15 s. Quadruple sets of four standards containing 10ng/μl, 1ng/μl, 100pg/μl and 10pg/μl domestic cat DNA respectively as well as one blank were amplified with the DNA samples of unknown quantity. We quantified the samples with STEPONE SOFTWARE v2.2 (Applied Biosystems).

To assess introgression levels and recognize individuals, we developed 96 Fluidigm SNPtype™ Assays for SNP genotyping (Fluidigm, San Francisco, USA). The marker set contains nuclear SNP markers (Nussberger *et al.*, 2013), as well as mtDNA markers described by Driscoll et al. (2007): 75 nuclear markers with a F_{ST} - value (genetic differentiation index) between wildcats and domestic cats ranging from 0.6 to 1 are for introgression level diagnosis, 11 nuclear markers with F_{ST} - values < 0.5 and four mtDNA markers for recognition of individuals, four diagnostic mtDNA markers for maternal lineage assessment and two diagnostic Y-linked markers for sex determination and paternal lineage assessment. Assay primers and sequences used to design them are shown in **Online Resource 1**.

Fluidigm SNP genotyping is analog to the Amplifluor genotyping system (for details see Morin & McCarthy 2007). In a first step, two pre-amplification primers (LSP=Locus Specific Primer and

STA=Specific Target Amplification primer) amplify the target region containing the SNP to be genotyped. Secondly, an additional PCR amplifies the targeted SNP region, using the LSP and two fluorescently labeled Allele Specific Primers ASP1 and ASP2. Finally, the SNP genotype is then determined by measuring the fluorescence intensity of both alleles. All 96 SNPs are pre-amplified simultaneously in one multiplex PCR, for each sample separately, on a Veriti Thermal Cycler (Applied Biosystems), with following conditions: hold at 95°C for 15min, 14 cycles at 95°C for 15sec and 60°C for 4min. The second PCR is performed on a Fluidigm 96.96 Dynamic Array (SNP chip), where the reactions occur in separate nano-wells for each SNP and sample combination, allowing simultaneous genotyping of 96 samples at 96 SNP loci. This PCR is performed on a BioMark HD System (Fluidigm), with following PCR cycling conditions: 50°C 2min, 70°C 30min, 25°C 10min, 95°C 5min; four touchdown cycles: 95°C 15sec, from 64°C to 61°C 45sec, 72°C 15sec; 34 additional cycles: 95°C 15sec, 60°C 45sec, 72°C 15sec, 1 cycle 20°C 10sec (for details see Fluidigm Genotyping User Guide).

We genotyped blood and tissue samples of 20 cats following the manufacturer's SNP genotyping protocol (Fluidigm Genotyping User Guide). For hair samples, we modified the protocol as follows. In the pre-amplification step, we used 2 or 4μl genomic DNA extraction solution, to increase the total number of DNA copies in the reaction above an a priori threshold of 50pg DNA per reaction. DNA was pre-amplified using 4μl QIAGEN Master Mix 2x, 0.8μl Specific-Target-Amplification primer pool and 1.2μl molecular grade water. The pre-amplification PCR product was diluted in 1:10. The number of additional cycles in the second PCR protocol was increased from 34 to 46. We included eight reference individuals (two domestic cats, two wildcats, one first generation hybrid and one backcrossed wildcat) and eight no template controls (NTC, for fluorescence plot normalization) in each chip. Genotypes of the reference individuals were known from previous genotyping based on Sanger sequencing. Fluorescence plots for each SNP were provided by Fluidigm SNP Genotyping Analysis software. All plots were checked visually and corrected manually for errors such as NTC with fluorescence values >0.1 or clusters which did not make sense in accordance to our reference samples. Except for the reference samples, we were naive to the true genotype of the samples during manual correction.

We tested the accuracy of our SNP genotyping assays by comparing the genotypes generated by Sanger sequencing and by Fluidigm for 17 blood or tissue samples. We calculated the genotyping error rate as the number of mismatches between Sanger genotype and Fluidigm genotype, divided by the total number of diploid markers genotyped with both methods. To estimate the rate of allelic dropout and false alleles (Pompanon et al. 2005), we assumed that the genotyping based on Sanger sequencing (Nussberger et al. 2013) showed the true genotype of an individual.

We genotyped four cats from which we had blood or tissue (high quality) samples as well as hair (low quality) samples to test whether our SNP assays yield reliable genotypes for low quality DNA samples. We analysed independently four hairs from each of the four individuals. For two individuals, we further duplicated these four low quality samples from the DNA extraction onwards, thus

generating 24 hair genotypes. We compared genotypes of high and low quality samples, both generated using the 96 Fluidigm SNPtype™ Assays as previously defined. We calculated the error rate in the genotypes from low quality samples using the genotype of the high quality sample as reference (genotypes are shown in **Online Resource 2**). Here we defined the error rate as the number of loci with mismatches between the high and low quality sample genotype divided by the total number of diploid loci genotyped. The proportion of false alleles was estimated as the number of homozygous loci in the reference genotype which were called as heterozygote in the hair genotype divided by the number of homozygote loci in the reference genotype. The proportion of allelic dropout was estimated as the number of heterozygous loci in the reference genotype which were called as homozygote in the hair genotype divided by the number of heterozygote loci in the reference genotype.

Finally, we checked whether the errors in the 24 hair genotypes affect assessment of identity and introgression levels. We used GIMLET (Valière 2002) to recognize individuals. Here, we considered an individual as recognized when at least 95% of all examined SNP genotypes of two samples were identical. We assessed individual introgression level based on 72 diagnostic nuclear SNP markers and using Bayesian model-based clustering by computing posterior probabilities for six different hybrid classes (two parentals, hybrid of first and second generation and two backcrosses) in NEWHYBRIDS (Anderson & Thompson 2002). We checked whether the hybrid class attributed to each of the hair genotypes were consistent within individuals. As a further control, we checked whether the hair genotypes of one individual lead to the same hybrid class as the tissue genotype.

Results and discussion

The here presented SNP genotyping method is reliable, even in samples of low quantity and quality, since genotyping error rates in single hair samples were low and did alter neither identity nor introgression level assessment. However, a minimal amount of genomic DNA of about 200pg is recommended. We believe this genotyping method is applicable to detect introgression in wildcats, based on non-invasive samples.

Four out of 17 individual Fluidigm genotypes based on high quality samples contained errors when compared to Sanger genotypes (Table 1a). Based on 816 pairwise comparisons of one locus genotype between Sanger and Fluidigm, the genotyping error rate per locus was 0.9%. Further, SNP genotypes were consistent between the four hair samples and the reference sample for all four individuals analysed (Table 1b). Overall, genotyping error rate per locus was 1.6%. Non-called loci were the most commonly observed error type. In the 16 hair-genotypes having at least 200pg DNA in STA pre-amplification, the overall error rate was 0.7%, allelic dropout was not observed, false alleles occurred in 0.1% of all homozygous SNP callings and non-called loci occurred in 0.6% of all SNP loci. The here observed error rates are somewhat below error rates estimated in studies using non-invasive sampling summarized by Valière et al. (2007). In Morin et al. (2001), PCR failures drastically

increased below 100pg in orang-utan hair and faecal samples. Thus, accurate quantification of samples is crucial to anticipate genotype quality (Morin et al. 2001; Beja-Pereira et al. 2009).

The high number of SNP markers and the low genotyping error rates in hair samples allow an accurate assessment of identity and introgression level. GIMLET attributed all except one hair sample to the correct individual out of the four genotyped individuals (**Online Resource 3**). Sample HK87_1, with 73pg of genomic DNA in the STA, had only 92% percent of identical loci with the other three hair genotypes from this individual and was thus considered as not correctly identified. The four DNA extractions from single hairs of the same individual always led to the same hybrid category as the reference genotype with a minimum posterior probability >0.99, even in the samples with the highest number of observed errors (**Online Resource 4**). The high accuracy of the introgression level assessment presented here was previously demonstrated (Nussberger et al. 2013) and mainly relies on numerous independently inherited diagnostic SNP markers with a strong differentiation in allele frequencies between wildcats and domestic cats. Thus, the introgression level in wildcat populations can now be assessed without invasive sampling and with more statistical power than shown in previous studies (Oliveira et al. 2008; Hertwig et al. 2009; Say et al. 2012). This represents a major improvement in conservation of the European wildcat, since representative DNA sampling from this elusive species relies mostly on non-invasive sampling.

An additional challenge when dealing with non-invasive sampling is the accurate identification of the studied species. For example, Monterroso et al. (2013) showed that accuracy of wildcat scat identification was low (11.5%) when based on morphology of scat alone. Thus it is worth including genetic identification in non-invasive studies (Oliveira et al. 2010). With the method presented here, species identification is assured by the use of cat specific primers in a quantitative real-time PCR. A preliminary test showed that applying these primers to high quality blood or tissue samples (20ng/μl) of human (*Homo sapiens*), squirrel (*Sciurus vulgaris*), stone marten (*Martes foina*), pine marten (*Martes martes*), European badger (*Meles meles*), brown hare (*Lepus europaeus*), raccoon dog (*Nyctereutes procyonoides*), European lynx (*Lynx lynx*) and red fox (*Vulpes vulpes*) did not yield any PCR product exceeding a concentration of 2pg/μl. Thus, we concluded that hair samples from other species than *Felis silvestris* are effectively eliminated prior to the following SNP assay, which consequently gets more efficient and cost effective.

In conclusion, the presented method allows simultaneous genotyping 96 SNP markers in 96 samples even with DNA of low quality and quantity. This protocol is suitable for non-invasively collected hair samples and can further be applied to other low quality DNA samples, such as faeces or historical specimens. The SNP chip presented here will help conservationists to monitor the introgression rate in wildcat populations based on non-invasive sampling and thus to better understand the process of hybridization.

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Table 1: Genotyping errors in cats (*Felis silvestris*) with Fluidigm SNP types assays when evaluating a) Fluidigm genotypes with Sanger sequencing genotypes as reference and b) hair sample genotypes with tissue sample genotypes as reference. DNA input quantity for specific target amplification is given in pg (>10 ng if not indicated). Tot.nr ref loci = Total number of reference loci, Tot.nr hetero loci = Total number of heterozygote loci, Tot.nr Errors = sum of occurrences of allelic dropout, false alleles and non-called loci. The percentage of allelic dropout (%AD) was calculated using Tot.nr hetero loci, the percentage of false alleles (%FA) using Tot.nr homozygote loci (Tot.nr ref loci - Tot.nr hetero loci).

Comparison	Names	DNA input	Tot.nr ref loci	Tot.nr hetero loci	Tot.nr errors	Allelic dropout	False alleles	Non-called loci	%Tot. nr errors	%AD	%FA	%Nonc alled loci
a) Fluidigm	HK080		39	4	0	0	0	0	0	0	0	0
versus	HK083		38	6	0	0	0	0	0	0	0	0
Sanger	HK086		45	3	0	0	0	0	0	0	0	0
	HK087		46	8	0	0	0	0	0	0	0	0
	HK088		39	2	0	0	0	0	0	0	0	0
	HK089		40	6	0	0	0	0	0	0	0	0
	HK092		38	1	0	0	0	0	0	0	0	0
	WK002		70	6	0	0	0	0	0	0	0	0
	WK017		49	2	3	0	3	0	6.1	0	6.4	0
	WK024		44	24	1	1	0	0	2.3	4.2	0.0	0
	WK026		45	25	1	0	1	0	2.2	0	5.0	0
	WK033		68	5	0	0	0	0	0	0	0	0
	WK036		48	5	0	0	0	0	0	0	0	0
	WK054		44	38	2	0	2	0	5	0	33.3	0
	WK058		62	2	0	0	0	0	0	0	0.0	0
	WK068		62	2	0	0	0	0	0	0	0	0
	WK077		39	1	0	0	0	0	0	0	0	0
	Overall a)		**816**	**140**	**7**	**1**	**6**	**0**	**0.9**	**0.7**	**0.9**	**0**
b) hair	WK145_4_r	680	83	16	0	0	0	0	0	0	0	0
versus	WK145_4	680	83	16	0	0	0	0	0	0	0	0
tissue/blood	WK145_3_r	400	83	16	0	0	0	0	0	0	0	0
	WK145_3	400	83	16	0	0	0	0	0	0	0	0
	WK145_2_r	280	83	16	0	0	0	0	0	0	0	0
	WK145_2	280	83	16	0	0	0	0	0	0	0	0
	WK145_1_r	220	83	16	0	0	0	0	0	0	0	0
	WK145_1	220	83	16	3	0	0	3	3.6	0	0	3.6

	WK014_4_r	280	83	24	0	0	0	0	0	0	0	0
	WK014_4	280	83	24	0	0	0	0	0	0	0	0
	WK014_3_r	200	83	24	1	0	0	1	1.2	0	0	1.2
	WK014_3	200	83	24	1	0	1	0	1.2	0	1.7	0
	WK014_2_r	200	83	24	1	0	0	1	1.2	0	0	1.2
	WK014_2	200	83	24	1	0	0	1	1.2	0	0	1.2
	WK014_1_r	120	83	24	1	0	0	1	1.2	0	0	1.2
	WK014_1	120	83	24	0	0	0	0	0	0	0	0
	WK006_4	246	83	21	1	0	0	1	1.2	0	0	1.2
	WK006_3	179	83	21	0	0	0	0	0	0	0	0
	WK006_2	101	83	21	1	1	0	0	1.2	4.8	0	0
	WK006_1	70	83	21	6	1	0	5	7.2	4.8	0	6.0
	HK087_4	304	83	15	1	0	0	1	1.2	0	0	1.2
	HK087_3	185	83	15	2	1	1	0	2.4	6.7	1.5	0
	HK087_2	164	83	15	3	1	1	1	3.6	6.7	1.5	1.2
	HK087_1	73	83	15	9	5	2	2	10.8	33.3	2.9	2.4
	Overall b)		**1992**	**464**	**31**	**9**	**5**	**17**	**1.6**	**1.9**	**0.3**	**0.9**

Chapter 3

Monitoring introgression in European wildcats in the Swiss Jura

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Conservation Genetics

Abstract

Introgression is an important evolutionary force, which can lead to adaptation and speciation on one hand, but also to genetic extinction on the other extreme. Introgression is a major conservation concern especially when domestic species reproduce with rarer wild relatives. Hence, monitoring introgression in natural populations subjected to hybridization is crucial to elucidate the threat represented by introgression. Here, we assessed introgression rates between wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis silvestris catus*). We monitored a wildcat population in the Swiss Jura Mountains based on systematically and non-invasively collected hair samples. We found 21% admixed individuals based on 68 diagnostic nuclear SNP-markers, corresponding to a migration rate from domestic cats to wildcats of 0.02 migrants per generation. In contrast, gene flow from wildcats into domestic cats was negligible. Haphazard sampling of the same wildcat population, mostly via road kills, led to similar results. Hybridization can occur between wildcat male and domestic cat female as well as vice versa and, based on the occurrence of backcrosses, both female and male F1-hybrids seem viable and fertile. The observed hybridization pattern may indicate an expanding wildcat population with introgression as a byproduct of this expansion.

Introduction

Introgression is an important evolutionary force, defined as the flow of genes between taxa through hybridization beyond the first generation of hybrids. It can lead to adaptation or speciation (Barton 2001; Grant et al. 2004; Mallet 2005; Arnold 2006; Baack & Rieseberg 2007; Mallet 2007). However, introgression is commonly thought to have a negative effect on rare and endangered species, because it can lead to genetic extinction (Rhymer & Simberloff 1996; Simberloff 1996). Introgression is a major concern especially when the source of hybridization is anthropogenic, that is, when domestic species reproduce with wild relatives (crops and wild forms: Ellstrand et al. 1999; dogs and wolves: Randi & Lucchini 2002; cattle and bisons: Halbert & Derr 2007; domestic and wild American mink: Kidd et al. 2009; pigs and wild boars: Goedbloed et al. 2013). Hence, for conservation purpose, it is crucial to monitor introgression in natural populations and assess the level of threat it represents for the species.

The European wildcat (*Felis silvestris silvestris*) is thought to be threatened by introgression with domestic and feral cats (*Felis silvestris catus*), as mentioned in the Red List of the International Union for Conservation of Nature (IUCN) as well as in the Red Lists of several countries (BUWAL 1994; ICNB 2004; Haupt et al. 2009; Driscoll & Nowell 2010). *Felis silvestris* is listed as a species of community interest in need of strict protection in the European Directive 92/43/EEC (Annex IV). Wildcats are known to hybridize with domestic and feral cats in several regions (Scottland: Beaumont et al. 2001; Italy: Randi et al. 2001; Hungary: Lecis et al. 2006; Iberic Peninsula: Oliveira et al. 2008a; Germany: Hertwig et al. 2009; France: O'Brien et al. 2009). Wild- and domestic cats are potentially hybridizing for over 2000 years, since Romans brought domesticated cats into the distribution range

of the European wildcat (Faure & Kitchener 2009). However, introgression of domestic genes into the wildcat gene pool was hardly detectable so far because morphologic and genetic methods failed to reliably recognize hybrids beyond the first generation (Devillard et al. submitted 2013). In addition, it is challenging to obtain an unbiased sample of a wildcat population. Sampling from road kills might not reflect a representative sample of the free-ranging population. Wildcats, hybrids and domestic cats might not be equally often killed on roads. In addition, hybrids with some domestic morphological traits may not be sampled, since a priori only the nationally protected wildcats are of interest to gamekeepers collecting the road kills. Hence, a systematically collected sample, without pre-selection through road or morphology, might better reflect the cat population. Such a sample can be obtained using lure stick hair-traps (Kéry et al. 2011). However, genotyping hair samples, or other samples of low DNA quality and quantity, is difficult (Gagneux et al. 1997; Vigilant 1999). Recent methodological advances alleviate these issues. First, introgression can now be recognized reliably, since a set of single nucleotide polymorphism (SNP) markers that are highly differentiated between wildcats and domestic cats has been developed (Nussberger et al. 2013). In addition, a SNP-genotyping method has been optimized to reliably amplify from single hairs (Nussberger et al. submitted 2013). Here, we use these SNP-genotyping methods to assess introgression rates in the wildcat population of the Swiss Jura region, based on two contrasting population samples: a non-invasively and systematically collected sample of hair and a haphazardly collected sample set of mostly road kills.

Materials and Methods

Sample collection

We had two datasets to estimate introgression in wildcat population of the Swiss Jura: a systematically collected hair sample set (monitoring samples) and randomly collected sample set (haphazard samples) which originated mostly from road kills.

The monitoring samples were hairs, collected non-invasively by gamekeepers, hunters and ourselves during the winters 2008/09 and 2009/10 using lure stick hair-traps, bated with valeriane and sampled every two weeks, five times in total. The hair traps were placed on a regular grid of 5x5 km covering the entire Swiss Jura region (3'719 km^2, 152 sites), the known core distribution range of wildcats in Switzerland (Nussberger et al. 2007). The sampling effort was intensified in the cantons of Geneva and Basel-Land, allowing delimiting the western and eastern edges of wildcat distribution in Switzerland more precisely. Sites without forest or within human habitations were excluded, since we expected to find mostly domestic cats in such sites. Three sticks were placed within each site (grid cell), that is, a surface of 1km^2, at least 50m inside the forest. Hairs from every stick and collecting date were separately collected in 10x15cm plastic bags (Minigrip) containing a 5g silicagel Tyvek packet (Dry & Safe GmbH) and stored in a freezer at -80°C about three days after collection in the field until further analysis.

Haphazard samples, dating from 2000 to 2013, were collected by the Centre for Fish and Wildlife Health of Berne, Switzerland, National History Museums and gamekeepers. This haphazard sample set contained 58 tissue samples and 14 hair samples (supplementary Table 1). Seventeen sampled cats were exhibiting obvious domestic morphological criteria, while the remaining samples were morphologically wildcats.

DNA extraction and quantification

Tissue samples were extracted using the DNeasy Blood & Tissue kit (Qiagen). Hair samples were extracted with the Sample-to-SNP-kit (Applied Biosystems) using a modified protocol (Nussberger 2013b). We placed each hair root singly into a 0.2ml PCR tube, added 9µl Lysis Solution and placed the tube in a thermocycler at 75°C for 10 min and 95°C for 4 min. Finally, we added 9µl Stabilization Solution.

DNA was quantified with quantitative real-time-PCR on a StepOnePlus instrument (Applied Biosystems). PCR contained 2µl DNA, 10µl FastStart Universal SYBR Green Master (ROX) 2x (Roche Applied Science), 6.64µl molecular grade water, 0.16µl BSA, 0.6µl forward and 0.6µl reverse cat specific cMyc primer of 10µM (F: ACGCACAACGTCTTGGAAC; R: TGGCCTTTTTAAGGATCACC). Initial incubation was set to 10 min at 95°C, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min (Nussberger et al. submitted 2013). This quantification step enabled us to exclude hair samples without sufficient DNA for accurate genotyping and hairs from other species such as the pine marten (*Martes martes*).

Genotyping

SNP-markers, genotyping assays and method were described in our previous work (Nussberger et al. 2013; Nussberger et al. submitted 2013). Briefly, we genotyped all our samples using 96.96 Fluidigm SNP genotyping arrays (SNP chips). The chip contained 75 nuclear markers with a F_{ST} - value (genetic differentiation index) between wildcats and domestic cats ranging from 0.6 to 1 for introgression diagnosis (75 diagnostic markers), 11 nuclear markers with F_{ST} - values < 0.5 and four mtDNA markers for individual recognition (15 identity markers), four diagnostic mtDNA markers for maternal lineage assessment (four female markers) and two diagnostic Y-linked markers for sex determination and paternal lineage assessment (two male markers). We replaced the assay Fst03_SNP149, which was not working in the previous study (Nussberger et al. submitted 2013) by SNP189 (C/T, locus specific primer: GACAATGAGCAAGGCAGGCA, specific target amplification (STA) primer: GTCTAATCAACCCAATACCACCC, allele specific primer (ASP) 1: ATGATGGCTCGACCAGAAGTTAG, ASP2: ATGATGGCTCGACCAGAAGTTAA).

We only genotyped samples with an estimated DNA amount of more than 0.005ng/µl, since below this threshold genotyping usually failed in preliminary tests. For some hair samples, we initially amplified the whole genome (WGA) prior to specific target amplification and genotyping, using a single cell WGA kit (WGA4 Sigma Aldrich). However, WGA did not improve hair genotyping success compared

to direct specific target amplification. Thus, we skipped the WGA for the majority of the hair samples. We used 2, 4 or 10μl DNA as input quantity into the specific target amplification (STA) to have at least 50pg DNA in the reaction. When using 10μl DNA, we increased the STA-PCR reaction volume to 21μl, by adding 10μl Qiagen Master Mix 2x and 1 ul STA-primer mix. These PCR products were diluted 1:5 prior to the SNP-chip genotyping PCR.

We excluded nine markers yielding unclear genotyping clusters (Fst01_SNP033; Fst33_SNP152; Fst31_SNP126; Fst37_SNP066; Fst45_SNP153; SNP109; SNP198; ID01_SNP134i; ID07_SNP144i) as well as individual genotypes with more than ten missing values (no calls) for downstream analysis. We also excluded genotypes from monitoring samples which had less than 0.2 ng DNA input, if another hair sample from the same collection bag contained more than 0.2 ng DNA and yielded a similar genotype of the two male markers and the same mtDNA haplotype.

Individual identity assessment for hair samples

To ensure that we do not count a genotype from the same individual multiple times, we assessed identity of each non-invasively collected hair sample using CERVUS 3.0.3 (Kalinowski et al. 2007). We allowed up to 5 mismatches between two genotypes before we attributed them different identities. This threshold was defined based on the preliminary analysis of quality control repeats. This quality control consisted in independently genotyping twice a set of 30 hair samples, 25 starting from STA and five starting from SNP-chip genotyping.

Introgression assessment

We assessed introgression between wildcats and domestic cats using Bayesian models.

First, we estimated the membership proportion of each individual to the wildcat and domestic cat populations, using Structure (Pritchard et al. 2000). We used an admixture model assuming k=2 populations and applied 100’000 iterations after 10’000 burn-in steps. The genotypes of 68 diagnostic markers from the monitoring and haphazard samples were analyzed both independently and pooled.

Second, we assessed the genealogical class for each individual, using NewHybrids (Anderson & Thompson 2002), allowing for six possible classes: wildcat (Wc), domestic cat (Dc), first generation hybrid (F1), F2 (offspring of F1xF1), backcross into wildcat (BxW) and backcross into domestic cat (BxD). In addition, a further analysis was performed allowing two more classes: third generation backcrosses into Wc (BxWxW) and Dc (BxDxD) respectively. We reran NewHybrids with the monitoring samples exclusively to estimate the distribution of the π-value, that is, the posterior probability frequency distributions of all the different genealogical classes present in the free-ranging cat population (Anderson & Thompson 2002).

Third, we inferred the migration rates per generation *m*, that is, the proportion of gene flow from the domestic cat population into the wildcat population and vice versa, based on 68 diagnostic markers, using BayesAss 3.0.3 (Wilson & Rannala 2003). The following mixing parameters were applied:

migration rates dM=0.1, allele frequencies dA=0.2, inbreeding coefficients dF=0.2. The Monte Carlo Markov Chains were iterated 5'000'000 with a burn-in of 1'000'000 and sampling interval being 1'000. We inferred the migration rates for each sample set independently and for all samples pooled. To increase proper mixing of the chains in the pooled dataset, we further modified the mixing parameters to dA=0.15 and dF=0.1.

Results

We collected hair samples in a total of 334 bags containing between one and 20 hairs from 105 sites. Seventeen sites could not be sampled, e.g. because of too high snow cover, whilst at additional 30 sites, no hairs were found (Figure 1).

We quantified the nuclear DNA concentration of 669 monitoring hair samples. 159 hairs (24%) contained more than 0.005ng/μl and thus qualified for genotyping. The observed low success rate can be explained by the fact that many hairs were likely not from cats and that several cat hairs had degraded roots (in telogen phase), leading to a too low amount of nuclear DNA in a single hair (Vigilant 1999).

We excluded 22 genotypes because they contained more than ten missing values (no calls).

Individual identity assessment for monitoring samples

CERVUS identified 75 individual genotypes among the 159 samples. Genotypes obtained from different hairs from the same sampling bag were attributed to a single individual, with seven exceptions. Six times two domestic cats and one time two wildcats of different sexes left hairs at the same lure-stick at the same collection session. No individual was found at more than one site. Detailed information on all monitoring samples is given in supplementary Table 2 (location, collection day, identity, genotype).

Introgression assessment

The genetic admixture analyses with Structure and NewHybrids revealed several individuals with an admixed genome in both sample sets (Figure 2).

NewHybrids categorized the 75 cats from the monitoring sample as 15 wildcats, four backcrosses into wildcats and 56 domestic cats (all posterior probabilities > 99.9%). The frequency distribution of the mixing proportions π of individuals of different genealogical classes indicates that the monitored cat population is composed of roughly 20% wildcats, 5% backcrosses into wildcats, 74% domestic cats and 1% of the other categories, F1, F2 and backcrosses into domestic cats (Figure 3). One backcross into wildcat was classified as third generation backcross in the analysis allowing also this additional category. Three of the 15 wildcats had mtDNA of the domestic type. Three out of four backcrosses were males and all three carried a domestic Y chromosome. Hence, 21% of the sampled individuals

of the wildcat population showed signs of recent introgression from the nuclear genome of the domestic cat. When also considering mtDNA introgression, the rate of introgressed individuals increased to 37%. In the domestic cat population, no signs of introgressed wildcat genes were found.

For the 72 haphazard samples, NewHybrids detected 34 wildcats, twelve backcrosses into wildcat (BxW), one F1, one F2, two backcrosses into domestic cats and 24 domestic cats. Individuals had over 95% posterior probability of belonging to their attributed genealogical class with two exceptions. One Wc had a posterior probability of 35% to be a BxW and one BxW had a posterior probability of 34% of being a wildcat, suggesting these individuals might be third-generation backcrosses. This was confirmed by the analysis with eight genealogical categories. In that analysis a further eight BxW and two Wc were also re-classified as third generation backcrosses. Twelve of 34 wildcats carried a domestic mtDNA. Hence, 29% of the wildcat individuals showed signs of introgression, 54% when including individuals where only the mtDNA showed evidence of introgression. One backcross into wildcat and two third-generation backcrosses were found in the southern Jura, all other admixed individuals were found in the northern region (Figure 1). Note, however, that more samples were collected in the northern region.

The migration rate *m* from domestic cats to wildcats per generation was estimated as 0.0161 (Standard Deviation=0.0154) individuals per generation in the monitoring sample set, 0.0347 (SD=0.0146) in the haphazard sample set and 0.0218 (SD=0.0102) when both datasets were pooled (Table 1). The migration rate in the opposite direction was lower in all datasets with values between 0.004 (SD=0.0039) and 0.0121 (SD=0.0118).

Discussion

We found widespread evidence of introgression from domestic cats into wildcats in the Swiss Jura. Hybridization can occur between wildcat male and domestic cat female as well as between wildcat female and domestic cat male, since we find backcrosses into wildcats with domestic Y or domestic mtDNA. Hence, both female and male F1-hybrids appear to be viable and fertile. Migration rate was estimated to be about 0.02 domestic migrants per generation into the wildcat population of the Swiss Jura. The strength of the present study resides first in the diagnostic panel of autosomal, Y-linked and mitochondrial SNP-markers, secondly in the reliable genotyping of single hairs and finally in the systematic sampling of the population in a short time frame based on two independent sampling regimes.

Population admixture

The introgression rate measured in the Swiss Jura is in the range of the rates observed in most of the surrounding countries of Western Europe. The rates of hybrid wildcats found in the Swiss Jura, ranging from 21% to 54%, may seem relatively high compared to the rates found in other genetic studies about hybridization between wildcats and domestic cats: 14% was observed in Portugal

(Oliveira et al. 2008b), 8% in Italy (Lecis et al. 2006), 4% in eastern and 42% in western Germany (Hertwig et al. 2009) and 36% in France (Say et al. 2012). For further comparison, 10% hybrids were found in a wild boar (*Sus scrofa*) sample set (Goedbloed et al. 2013) and 4% and 5% in wolves (*Canis lupus*) in Portugal and Italy respectively (Verardi et al. 2006; Godinho et al. 2011). However, all these studies applied different genetic markers (e.g. microsatellites) and different hybrid threshold definitions. Some difference in hybridization rates may likely be explained by these methodological differences. To our knowledge this study is the first using diagnostic nuclear markers, complemented by mitochondrial and Y-linked markers. The markers used here outperform the microsatellites used so far in recognizing wildcat hybrids over the first generation (Nussberger et al. 2013). Thus, it is possible that more individuals could be recognized as hybrids in our study than in previous ones and this may explain the somewhat higher rate of hybrids in the Swiss Jura region. However, the percentage of animals with some hybrid ancestry may not be the best way to compare the occurrence of introgression between studies. A comparison of the migration rates per generation may be more relevant. In fact, the observed migration rate of 0.02 migrants per generation from domestic cats into wildcats in the Swiss Jura is somewhat lower than the one found in Portugal (m=0.064; Oliveira et al. 2008b) and slightly higher than in Germany (m=0.004 in Eastern and m=0.013 in Western Germany; Hertwig et al. 2009), but still in the same overall range. Nevertheless, introgression rates are not homogeneous over the whole distribution range of the European wildcat. Especially, introgression rates observed in Hungary are almost four times higher than the ones observed in Italy (Lecis et al. 2006), and introgression rates in Scottland are very high (Beaumont et al. 2001).

Gene flow seems to be mostly directed from domestic cats into wildcats rather than in the opposite direction. We only observed two backcrosses into domestic cats in the haphazard dataset (out of 26 domestic cats) and none in the monitoring set (out of 56). Obviously, we might miss some of them due to our sampling strategy, which favor wildcat rather than domestic cat sampling (road kill collection mainly if wildcat phenotype; hair collection sites outside human habitations and inside forests). Nevertheless, this bias should apply to backcrossed and pure domestic cats equally and the ratio between backcrossed and pure domestic cats remains very low. In addition, we did not find a wildcat mitochondrial haplotype in any of the domestic cats sampled. In contrast, we found many wildcats having a mitochondrial haplotype clustering with domestic cats. Several processes could explain the asymmetrical gene flow from domestic cats towards wildcats. First, this pattern could be explained by an expanding wildcat population into areas in which domestic cats are already present in higher densities. Indeed, expanding populations have lower density near the expansion front, and are prone to introgress with the locally well established population (Currat et al. 2008). This theory would be congruent with the observation of expanding wildcat populations in France (Say et al. 2012). Alternatively, the asymmetrical introgression pattern might also be explained by a sex-bias in either gene flow or hybrid survival or both. Indeed, we found several wildcats, also beyond third generation of hybridization, that have mtDNA from the domestic cat, indicating a possible ancient introgression on the female line. In contrast, we did not find any domestic introgression on the paternal line going further than the second generation of hybrids. Thus, domestic introgression might be more frequent on the female line, i.e. matings between domestic females and wild males might be more frequent

than vice versa, or male hybrids with introgressed domestic Y-chromosome might have a lower survival than hybrids with domestic mtDNA introgression. Such asymmetric hybridization pattern is common. For example, in polecats (*Mustela putorius*) and minks (*Mutela lutreola*), introgression is directed from minks into polecats and matings occur only between male polecats and female minks (Cabria et al. 2011). Matings between female wolves (*Canis lupus*) and male dogs (*Canis l. familiaris*) seem more common than vice versa (Hindrikson et al. 2012 and references therein). A directional and asymmetric introgression pattern was also found between two highly divergent lineages of field voles (*Microtus arvalis;* Beysard et al. 2012).

Geographic distribution of hybrids

In accordance with the population expansion hypothesis mentioned above, one could expect more hybrids in the periphery of the wildcat range. This is difficult to infer from our own data, since the whole Swiss Jura region reflects only the edge of a larger wildcat population. In Italy, wildcat-domestic cat hybrids were found at the periphery of their ecological range (Randi 2008), and the same was found for wolf-dog hybrids in Italy and Portugal (Verardi et al. 2006; Godinho et al. 2011). On the other hand, in France, wildcat-domestic cat hybrids were found throughout the main area of wildcat occurrence (Say et al. 2012). It would be interesting to compare locations of hybrids with human density maps. Indeed, where human density is high, density of domestic cats may also be high and, with it, possibilities of hybrid matings for wildcats.

Influence of sampling strategies

Achieving unbiased and representative sampling over a large geographical range is not trivial, and it is important to be aware of possible biases in sampling collection methods. Biases that affect wildcats, domestic cats and their hybrids similarly can be tolerated for estimating introgression rates, since the ratio between hybrids and non hybrids would then remain the same as without bias. Also, if known and quantifiable biases affect only one or the other group, data could be corrected for these biases. Problems arise when biases are not similar and not quantifiable between wildcats, domestic cats and their hybrids. Haphazard sampling of road kills is often used (Randi et al. 2001; Verardi et al. 2006; Hertwig et al. 2009), but might be biased for calculating introgression rates: Road kills from hybrids and backcrosses having a domestic phenotype are most probably not collected at all. On the other hand, hybrids might be overrepresented in such a sample set, if they are more at risk of getting killed on a road, e.g. because they get closer than wildcats to human habitations and thus to denser road networks (Germain et al. 2008; Klar et al. 2008). Another possible bias of haphazard sampling is a spatial one. Our haphazard dataset contains more samples from the northern part of the Jura than the southern part. It is tempting to conclude a higher density of wildcats in the north. However, it could well be that wildcats are simply more often found in the north, because the region is more densely populated than the south, meaning more potential road kill finders. In addition, people may be better informed about the need to collect samples from an endangered species like the wildcat in some areas than in others. These biases can be alleviated, if road kills are exclusively sampled by persons trained to apply the same collection scheme over a given geographical area (Say et al. 2012). Despite

all the potential biases of haphazardly collected samples, hybridization rates in both our systematically and non-systematically collected sample sets were comparable (21% and 29%) and the estimated migration rates of both sets had largely overlapping credible intervals. In any case, the presented non-invasive systematic survey has advantages over the haphazardly collected road kill samples. Especially, the intensity and timing of the sampling can be modified by the researcher in function of the needs of a given study, which is obviously not possible when relying on study objects getting killed.

Implications for conservation

For conservation purposes, one of the main questions is whether hybrids and introgressed individuals suffer from lower fitness. We did not find any evidence for or against lower hybrid fitness. Several individuals that were wildcats based on the nuclear markers had introgression on mitochondrial DNA. This can be seen as a sign of introgression further back in the female line, suggesting that introgressed individuals have reproduced successfully over many generations. In addition, we observed more than twice as many backcrosses as first generation hybrids. Considering three generations and the pooled data set, we find a ratio of 1:10:13 between first, second and third generation hybrids. In absence of selection and assuming a constant effective population size, we would expect a ratio of 1:2:4. However, the assumption of constant population size is likely to be violated. Thus, negative selection on hybrids cannot be inferred or ruled out with the present data.

The observed gene flow of 2% from domestic cats to wildcats may appear relatively low. However, even low introgression can lead to rapid evolutionary changes (Cavalli-Sforza et al. 1994). If we make the – rather unrealistic – assumptions that population size, migration rate and gene flow stay constant over time and that the effect of selection and drift is negligible relative to that of gene flow, a gene flow of 2% from domestic cats to wildcats could entirely replace the gene pool of wildcats within 263 generations (or 789 years assuming a cat generation time of three years), following the equation $m(n)=1-(1-m)^n$ where m=migration rate 2% and n=number of generation (Cavalli-Sforza et al. 1994). Nevertheless, several studies showed that introgression is not necessarily bad. Introgression may counteract effects of inbreeding, like in Darwin's finches (Grant et al. 2003) or can lead to rapid adaptive evolution, as in coyotes, gaining in size through hybridization with wolves (Kays et al. 2010). Hybridization can have positive effects, even when introgressed genes are domesticated, as shown in the Soay sheep (Feulner et al. 2013). In addition, introgression might simply be a byproduct of an expanding population, a selectively neutral process (Petit et al. 2004; Currat et al. 2008). Be this as it may, it remains a conservation concern to better understand how threatening introgression with domestic cats is to wildcats, based on the precautionary principle. This is especially true since it has been shown that introgression rates in wildcats can be much higher, as in Scottland (Beaumont et al. 2001) and Hungary (Lecis et al. 2006).

Here, we presented the results of a systematic wildcat monitoring, based on non-invasive hair sampling and using diagnostic nuclear markers. Our data are a baseline for further wildcat surveys. Monitoring introgression over broader space- and timescales will give more insight into the

introgression process. However, this alone will not be sufficient. To assess the extent of the threat to wildcats through introgression of domestic genes, we need more information on demographic parameters, such as population densities and fitness of hybrids.

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B.N., P.W., L.F.K. designed research; B.N. performed research and field work.

The authors declare no conflict of interest.

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Table 1: migration rates (*m*) per generation between domestic cats and wildcats, analyzed in BayesAss for monitoring and haphazard sample sets, separately and pooled. Bayesian model mixing parameters were: for migration rates dM=0.1, for allele frequencies dA=0.2 (0.15 in pooled set), for inbreeding coefficients dF=0.2 (0.1 in pooled set); MCMC with 5 mio iterations, 1 mio burn-in, sampling interval 1000. n= sample size, SD=standard deviation, CI=credible interval.

Sample set	**n**	***m* domestic into wild (SD)**	***m* domestic into wild** **95% CI**	***m* wild into domestic (SD)**	***m* wild into domestic** **95% CI**
monitoring	75	0.0161 (0.0154)	0.0000-0.0430	0.0057 (0.0057)	0-0.0169
haphazard	72	0.0347 (0.0146)	0.0061-0.0633	0.0121 (0.0118)	0-0.0352
pooled	147	0.0218 (0.0102)	0.0018-0.0418	0.0040 (0.0039)	0-0.0116

The supplementary Tables are published online in *Conservation Genetics*.

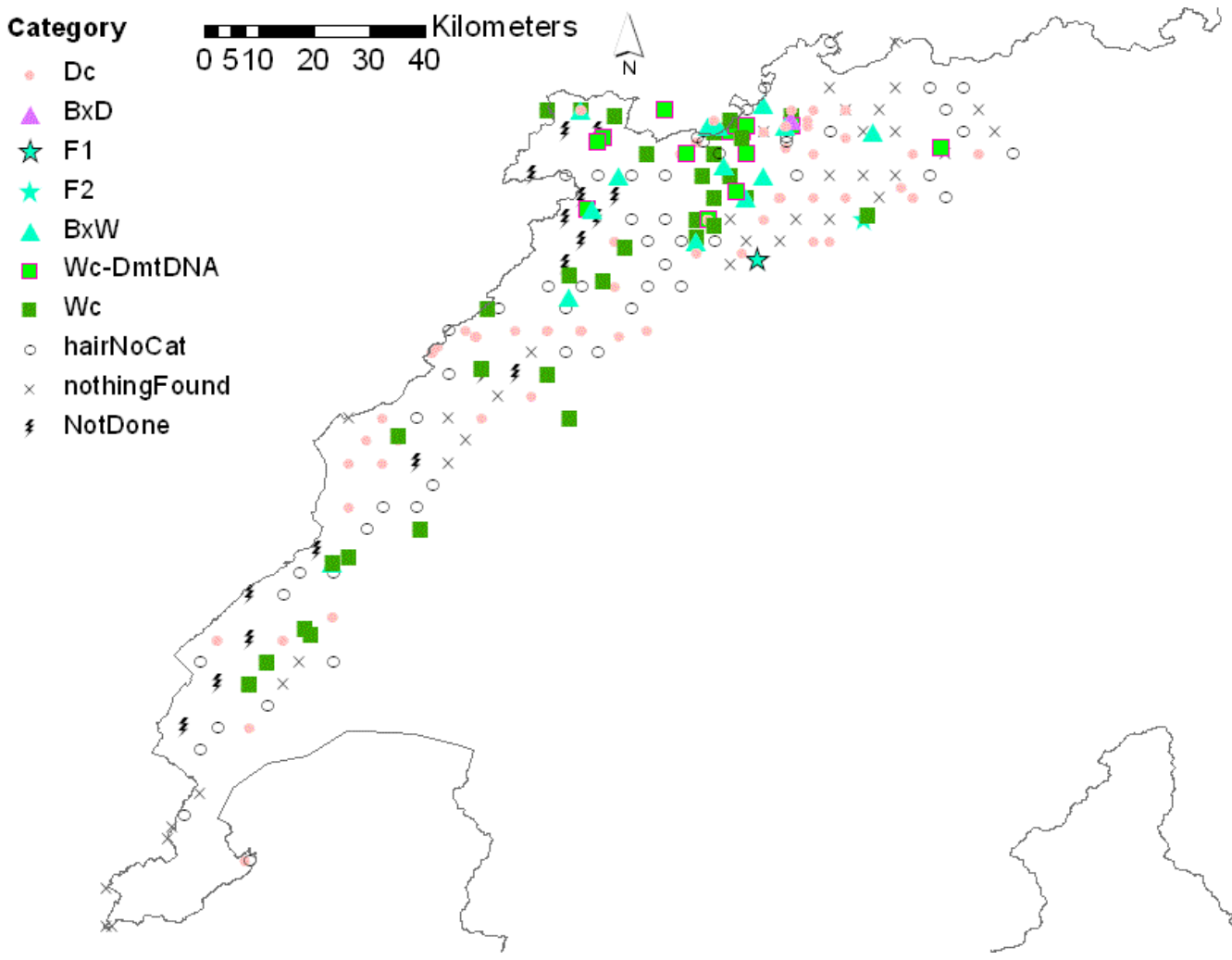

Figure 1: Sampling sites throughout the Swiss Jura (monitoring and haphazard samples). Wc=wildcat, Wc-DmtDNA=wildcat based on nuclear markers with mitochondrial DNA of domestic type, BxW=backcross into wildcat, F1=first generation hybrid, F2=offspring of F1xF1, BxD=backcross into domestic cat, Dc=domestic cat.

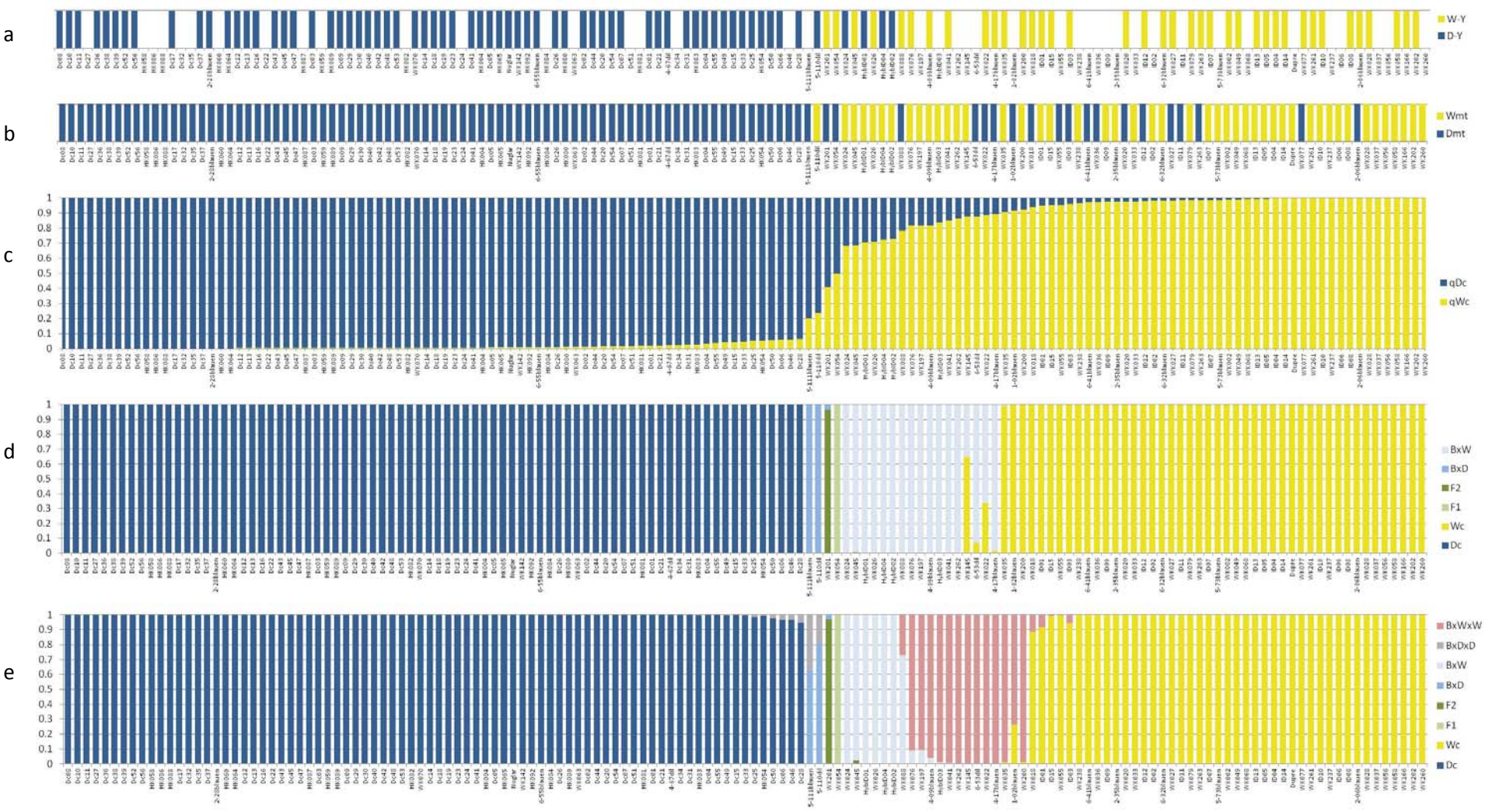

Figure 2: Proportion of genome from wildcats (yellow) and domestic cats (dark blue) for each individual based on Y marker (a) and mtDNA (b), as well as on 68 nuclear diagnostic markers in Structure (c). d) NewHybrids posterior probabilities of belonging to the different genealogical categories: domestic cat (Dc, dark blue), wildcat (Wc, yellow), backcross into Dc (blue), F1 (light green), F2 (dark green), backcross into Wc (light blue). e) same as d) but with two more classes: third generation backcrosses into Wc (rose) and into Dc (grey).

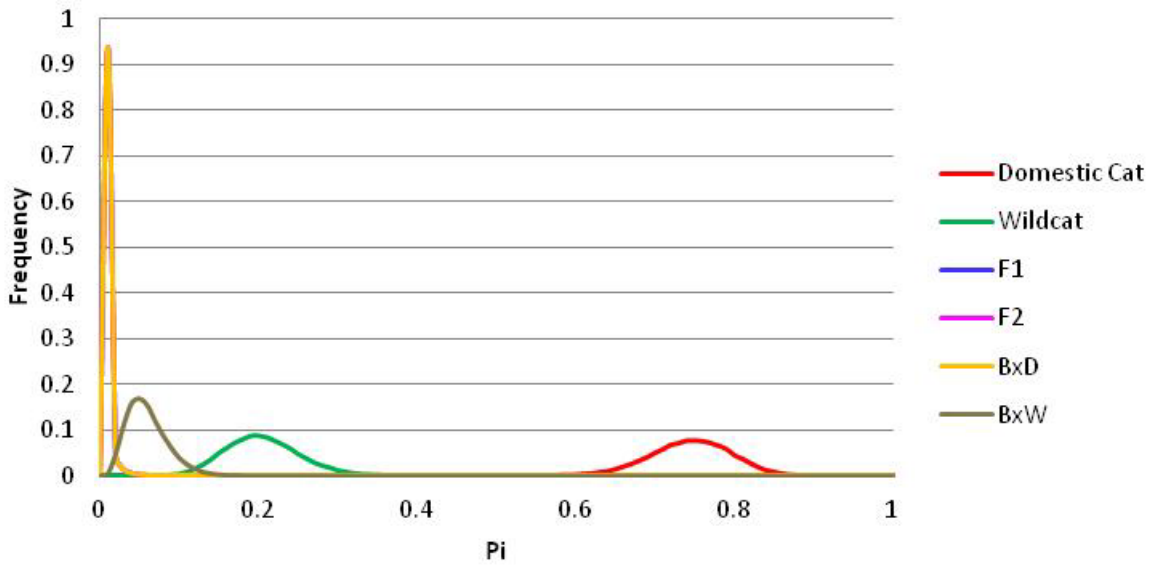

Figure 3: Proportions of the six genealogical classes in NewHybrids (π-value)

Chapter 4

Are wildcats reconquering areas once lost to domestic cats?

Beatrice Nussberger, Nicole Ponta, Lukas Keller

Abstract

Introgression between domestic and wild taxa is a conservation issue, because in the worst case, introgression could lead to the genetic extinction of the wild species. To assess how much introgression threatens a given species, it is crucial to understand the mechanisms leading to introgression. Here, we assessed the extent and direction of introgression within biparentally, paternally and maternally inherited genetic markers between European wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis silvestris catus*). Using Bayesian approaches, we analyzed 68 autosomal, two Y-chromosomal and four mitochondrial diagnostic single nucleotide polymorphisms, as well as a sequence of 384bp mtDNA, in a set of 491 wild- and domestic cats from France, Switzerland and Germany. We found 53 hybrids, mostly backcrosses, resulting in a migration rate into wildcats of 2% per generation. Introgression of maternally inherited mitochondrial markers was higher than paternally inherited markers. The mitochondrial haplotype most often introgressed into wildcats was the most frequent haplotype within the domestic cats. We found evidence for population growth in a well defined wildcat population of the Jura region. The observed hybridization patterns might be explained by a selectively neutral model and might indicate local expansion of the European wildcats.

Introduction

Understanding introgression mechanisms is crucial to species conservation because hybridization can threaten species (Rhymer & Simberloff 1996) especially when hybridization involves domesticated species (Allendorf et al. 2001). Introgression from domestic species can cause outbreeding depression and swamping of the genome in the wild species (Ellstrand et al. 1999). On the other hand, hybridization can also be an evolutionary force that may lead to adaptation and speciation (Grant et al. 2004; Seehausen 2004; Arnold 2006), or it can be a neutral phenomenon that occurs when a taxon invades an area occupied by another closely related taxon (Currat et al. 2008). In the absence of selection, the invasive taxon is predicted to be more introgressed than the local taxon, especially on markers having reduced gene flow. In species with sex-biased dispersal, the least-dispersing sex is predicted to have lower gene flow (Petit et al. 2004; Currat et al. 2008; Petit & Excoffier 2009). These predictions base on the different effective population sizes in taxon and sex. The invading taxon has lower densities than the local taxon, thus the directionality from local to invader. The least-dispersing sex has lower local effective population size compared to the most-dispersing sex, thus the asymmetry between both sexes.

European wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis silvestris catus*) are known to hybridize and produce fertile offspring (Beaumont et al. 2001; Randi et al. 2001; Lecis et al. 2006; Oliveira et al. 2008; Hertwig et al. 2009; O'Brien et al. 2009). Domestic cats were probably brought into European wildcat habitat through the Romans, about 2000 years ago (Faure & Kitchener 2009). Hence, hybridization between both subspecies potentially could have been occurring for many generations. The ancestor of the domestic cat, i.e. the African wildcat *Felis silvestris libyca,* and *Felis*

silvestris silvestris split only roughly 200'000 years ago (Driscoll et al. 2007). Reproductive barriers are expected to be low between taxa having such short divergence times (Mallet 2005). The International Union for Conservation of Nature IUCN considers hybridization with domestic cats a major threat to European wildcats (Driscoll & Nowell 2010). Nevertheless, there is some evidence that wildcat populations are presently expanding in Germany (Klar et al. 2008; Streif et al. 2012), France (Say et al. 2012) and Switzerland (Weber et al. 2010). Here, we aim to estimate introgression rates in a large set of free-ranging wildcats of Western Europe and increase the knowledge of the hybridization patterns between these two subspecies, especially in respect to the mode of inheritance of the introgressed genes. We hypothesize that introgression might well be a selectively neutral process, if we observe a directional introgression, where the expanding taxon is more introgressed than the local one, and a sexually asymmetric hybridization pattern, where the least-dispersing sex is more introgressed than the most-dispersing sex. Thus, we assessed the extent, direction and asymmetry of introgression within biparentally, paternally and maternally inherited genetic markers between European wildcats and domestic cats, by analyzing 68 autosomal, two Y-chromosomal and four mitochondrial diagnostic single nucleotide polymorphism (SNP) markers, as well as a sequence of 384 bp of the highly variable mitochondrial control region, in a set of 491 wild- and domestic cats from France, Switzerland and Germany.

Material and methods

We collected 491 tissue and hair samples from wildcats and domestic cats from different sources and countries (supporting information 1). Most of the samples dated from 1990 to 2013, but 22 samples were older and go back until 1915.

To assess introgression patterns of biparentally, maternally and paternally inherited markers, we genotyped 68 autosomal nuclear SNP-markers, four mtDNA SNP-markers and two SNP-markers on the SRY region on the Y chromosome. All these SNPs are highly differentiated or differently fixed between wildcats and domestic cats and thus diagnostic (Nussberger et al. 2013). We genotyped these diagnostic SNP-markers using 96.96 Fluidigm SNP genotyping arrays (SNP chips). Details about DNA extraction methods, diagnostic SNP-markers and genotyping methods were previously described (Nussberger et al. 2013; Nussberger et al. submitted 2013a; Nussberger et al. submitted 2013b).

We assessed nuclear introgression between wildcats and domestic cats using three Bayesian models. First, we assessed the nuclear admixture level of all samples with Structure 2.3.1 (Pritchard et al. 2000), assuming two populations (k=2), i.e. wildcats and domestic cats, and running the default admixture model over 10^5 MCMC iterations, after a burn-in period of 10^4 with correlated frequencies between populations. Second, we used NewHybrids 1.1 beta (Anderson & Thompson 2002) to determine the nuclear genealogical category of each individual, allowing for wildcat (Wc), domestic cat (Dc), first generation hybrid (F1), F2 (offspring of F1xF1), backcross into wildcat (BxW) and

backcross into domestic cat (BxD). We also ran NewHybrids with the same sample set allowing for two more classes: third generation backcrosses into Wc (BxWxW) and Dc (BxDxD). Finally, we estimated the migration rate between wildcats and domestic cats with BayesAss 3.0.3 (Wilson & Rannala 2003). We changed the parameter dM to 0.05 to assure proper mixing. To calculate the migration rate in modern wildcats, we selected only samples collected after 1990 upwards. We defined the domestic cat group as those individuals categorized by NewHybrids as Dc or BxD. All other samples were considered as belonging to the wildcat group. The programs Convert 1.3.1 (Glaubitz 2004) and Formatomatic 0.8.1 (Manoukis 2007) were used to convert formats of the input files between softwares.

To assess mtDNA haplotypes for a subset of 400 samples, we used the two primer pairs Lf15926 - Hf3 and Lf4 - DLH (Eckert et al. 2010) with annealing temperatures of 50° and 56°C respectively. These two primer pairs yielded two sequences of the mitochondrial control region of about 350 and 200 bp, respectively. Sequencing of the PCR products was performed using Big Dye Terminator v3.1 chemistry on a 3730 DNA Analyzer (Applied Biosystems). The sequences obtained were base called using Sequencing Analysis v5.1 (Applied Biosystems) and subsequently edited in Geneious Pro v5.5.6 (Drummond et al. 2009). All sequences were trimmed to the same length, removing only non-polymorphic sites. For each individual we then collapsed the two shortened fragments into one single sequence of about 384bp. Using Arlequin v3.5 (Excoffier & Lischer 2010) we inferred haplotypes considering only sites with less than 4% missing data, and we calculated a minimum spanning tree among them. The resulting network was drawn in Hapstar v0.5 (Teacher & Griffiths 2011).

We performed Fu's F_s neutrality test (Fu 1997) to see if there was any evidence that the wildcat population is growing, using Arlequin (Excoffier & Lischer 2010). For this analysis, we selected only the mtDNA sequences from individuals having a wildcat haplotype, to avoid biased results through the effect of domestic haplotype introgression. In addition, we restricted the samples to the wildcats from the Franco-Swiss Jura region (Figure 1, n=87), to avoid substantial population substructure in the sample. This population showed no substructure in a preliminary study with 21 microsatellites and 2600bp mtDNA sequences (Ponta 2012). We also performed a mismatch analysis with the same sample subset, to test for sudden population expansion, a test that is known to be very conservative (Ramos-Onsins & Rozas 2002).

Results

From the 491 analyzed cat samples, 53 (11%) were hybrids, based on 68 nuclear diagnostic SNP-markers (Table 1). The most common category of hybrids were backcrosses into wildcats, but all other hybrid classes were also found. The posterior probabilities were >0.95 for all but 12 samples. These 12 samples had their posterior probabilities increased above 0.95 in the second NewHybrids run allowing for two backcross generations. Ten of them changed categories: five Wc and five BxW changed into BxBxW. Results of both NewHbyrids runs are shown in supporting information 1.

These hybridization rates resulted in a migration rate from domestic cats into wildcats of 0.0176 migrants per generation (95% credible interval 0.009 - 0.026). Migration rate from wildcats to domestic cats was 0.007 (95% CI 0.000 - 0.015), which is significantly lower (dependent T-test for paired samples, $p<0.001$).

The known locations of wildcats and hybrids are shown in Figure 1. Hybrids seem concentrated at edges of wildcat distribution and around major cities, especially Basel and Freiburg in Breisgau.

Based on the four mitochondrial and the two Y-chromosomal diagnostic SNP-markers, the maternally inherited mtDNA was more often introgressed than the paternal Y chromosome, with 63 out of 481 and 15 out of 311 individuals being introgressed at the mtDNA and the Y chromosome, respectively ($\chi^2 = 14.566$, p-value = 0.0001, Table 1).

Within the 400 samples from which we sequenced mtDNA, we found 45 distinct haplotypes in two clusters (Figure 2). These clusters were attributable to domestic cats and wildcats, with 31 and 14 distinct haplotypes, respectively. Although roughly two times more wildcats than domestic cats were sequenced, haplotype diversity was higher in domestic cats than in wildcats. Forty-five of the 237 samples that classified as wildcats based on the autosomal SNPs had introgressed domestic mtDNA haplotypes, 39 of them carrying the haplotype Hap09 (Figure 2 and 3). This haplotype was by far the most common haplotype in domestic cats. Three haplotypes of the domestic cluster (Hap15, 41 and 49) were only found in wildcats or hybrids. F1, F2 and backcrosses into wildcats showed in total twelve different haplotypes (five of which were domestic), suggesting several recent hybridization events.

Fu's F_S was significantly negative ($F_S = -26.84833$, $p<0.0001$), suggesting that the wildcat population in the Jura region is growing. This tendency was confirmed by a mismatch analysis. The test of goodness-of-fit revealed a p-value of 0.474, suggesting that we cannot reject the null hypothesis of population expansion (Harpending's Raggedness index = 0.02776833, p = 0.815).

Discussion

We quantified the amount of gene flow between wildcats and domestic cats in France, Germany and Switzerland and discovered hybridization patterns which may support the hypothesis of introgression being a selectively neutral process coming along with a range expansion of wildcats. Diagnostic nuclear SNP-markers revealed ongoing hybridization over at least three generations. The migration rate from domestic cats to wildcats was of roughly 2%, which is in the range of what was found in other studies throughout Europe, with exception of Scottland and Hungary (Beaumont et al. 2001; Lecis et al. 2006; Oliveira et al. 2008; Hertwig et al. 2009; Say et al. 2012; Nussberger et al. submitted 2013b). The migration rate from wildcats to domestic cats was lower, suggesting a directional introgression. We observed a sexually asymmetric introgression, with more introgression into the female line, the one of the least-dispersing sex. Introgression of maternally inherited markers was

higher than introgression of paternally inherited markers. We found no Y-chromosome introgression going further back than the second generation of hybridization. In contrast, we found introgression of domestic mitochondrial DNA in cats nuclearly defined as wildcats. Mitochondrial introgression without apparent nuclear introgression is common and likely indicates more ancient hybridization events, as shown in several species (Bachtrog et al. 2006; Zielinski et al. 2013).

Most (87%) nuclear wildcats with introgressed mtDNA, i.e. wildcats with introgression dating back at least four generations, had the same haplotype Hap09 from domestic origin. In the more recent hybrids, i.e. F1, F2 and backcrossed wildcats, we observed 12 haplotypes, five being from domestic origin. Here again, the most frequent domestic haplotype was Hap09, with seven individuals carrying it, whereas the four other haplotypes were represented by only one individual each. This pattern is most parsimoniously explained by the high frequency of the haplotype Hap09 (34%) in the domestic cat population. In the absence of selection, the most frequent domestic type is expected to introgress most frequently, and thus it is most frequently detected in introgressed wildcats. Nevertheless, we may also expect that the ratio between domestic cats with haplotype Hap09 and domestic cats with haplotype different from Hap09, i.e. 34% : 66%, would be reflected in these wildcats with introgressed mtDNA, which is not the case here (87% : 13%). This might partially be explained by spatial variation in haplotype frequency. However, we cannot entirely rule out some positive selection on this haplotype Hap09, since it is so common in both, wildcats and domestic cats.

The two Y-chromosomal SNP-markers were not working optimally. We observed that several known females led to a fluorescent signal in the genotyping plot for one or both Y-SNP-markers. This is probably due to a cross-reaction of the fluorescently labelled primers in absence of the target DNA of the Y chromosome. Fortunately, we knew the sex of most of the samples and could correct for these errors. We also double-checked the domestic or wild origin of the Y in 78 samples (65 presumed males and 13 presumed females) with another Y marker, the SMCY-microsatellite (Nussberger et al. 2013). Unfortunately, the microsatellite fragment analysis failed to amplify any allele for 33 potential males. It is unclear whether this amplification failure was due to technical problems, or – less probably – due to erroneous initial sexing. Those SMCY-alleles that did amplify successfully were consistent with the Fluidigm markers with two exceptions, suggesting that our Y-chromosome results are reasonably reliable despite the technical issues in the SNP analysis. For two presumed males, however, SMCY indicated a domestic allele, whereas Fluidigm SRY-SNPs indicated a wild allele. These individuals were assumed to be of unknown sex in our analysis. Optimized diagnostic Y-markers would facilitate future analyses.

Wildcats were more often introgressed on maternally than on paternally inherited markers. Different patterns of introgression between maternally and paternally inherited genes are often observed (examples reviewed in: Petit & Excoffier 2009; Beysard et al. 2012). Since wildcats have male-biased dispersal, this result is consistent with a selectively neutral model, predicting more introgression on the least-dispersing sex, that is, the sex with lower intraspecific gene flow (Currat et al. 2008; Petit & Excoffier 2009). In addition, this hybridization pattern could be enhanced by asymmetric mating preference, i.e. by matings between wildcat males and domestic females being more common than

vice versa, a situation that has been observed for example in hybridizing savannah and forest elephants (Roca et al. 2005). However, in our rather small sample of first generation hybrids, we did not find evidence for such a behavioural reproductive barrier, since we found more F1 with a domestic father (five) than with a wild father (three). Another explanation for the asymmetric hybridization pattern is sex-biased survival of hybrids. Haldane's Rule predicts that if, in hybrids, one sex is rare, that sex will be the heterogametic sex (Haldane 1922). In the case of wildcats, this would mean a lower fitness of hybrid males. Y chromosomes would then have a lower chance to introgress than mtDNA. However, we did not observe a deficiency in hybrid males. The sex-ratio did not differ significantly in the hybridized (1.9) and non-hybridized cats (1.5; χ^2 = 0.985, p=0.321). Thus, we believe the pattern is best explained by sex-biased dispersal.

Introgression of organelle genes and directional introgression from one taxon to another is often observed after a range expansion (Currat et al. 2008). Petit et al. (2004) suggest that hybridization may be a mechanism of dispersal: gene flow between taxa can facilitate colonization of new areas. In the colonizing taxon, the more philopatric sex is replaced – at least temporarily – with the other taxon already present in the area, thus accelerating the invasion. Here, we found weak evidence that this could be the case in wildcats. First, wildcats were more introgressed on the maternally inherited mitochondria than on the paternal Y-chromosome, females being the more philopatric sex. Second, introgression into wildcats (1.7%) tends to be somewhat higher than introgression into domestic cats (0.7%). However, our sample collection is biased towards wildcat-like hybrids and against backcrosses into domestic cats morphologically resembling domestic cats, thus introgression into domestic cats might be underestimated. Nevertheless, this directionality from wildcats to domestic cats was also observed in a dataset that was obtained in a manner that is less susceptible to biases (Nussberger et al. submitted 2013b). Third, we could reject the hypothesis of constant population size in the wildcat population in the Franco-Swiss Jura, previously shown to be a single panmictic population. Further, there are other indications of wildcat population growth throughout Europe (Germany, references in: Klar et al. 2008; Switzerland: Weber et al. 2010; France: Say et al. 2012). Especially, in Baden-Württemberg, two road kills from 2006 and 2007 were the first evidence for wildcat presence in this region since the assumed local wildcat extinction in 1912 (Streif et al. 2012). The number of wildcat observations has increased over the last decades. However, we can't exclude an increase in the detection probability, following the increased presence of the wildcat in the media in this time period, at least in Germany and Switzerland. Finally, hybrids seem to concentrate at local edges of the wildcat distribution, which would be compatible with the hypothesis of an expansion. Although they need to be confirmed by more in depth analyses of several demographic parameters, all these observations – domestic mtDNA introgression into wildcats, directional introgression from domestic to wildcats, possible population growth and peripheral location of hybrids – would fit the theory of range expansion of wildcats into areas mostly occupied by domestic cats in the past decades. Currently, European wildcats from France, Switzerland and Germany might profit from the presence of domestic cats to expand their territories – expanding faster than without domestic cats.

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Table 1. Introgression of mitochondrial DNA (mtDNA) and Y chromosome markers in the six hybrid classes defined through autosomal nuclear markers (NewHybrids). Wc = wildcat, BxW = backcross into wildcat, F1 = first generation hybrid, F2 = offspring of F1xF1, BxD = backcross into domestic cat, Dc = domestic cat, qWc = proportion of membership to wildcat cluster (Structure), N = sample size, N m = number of males, D = domestic, W = wild. Introgressed individuals highlighted in bold; F1 and F2 cannot be introgressed per definition.

Hybrid Category	**qWc**	**N**	**N m**	**mtDNA D**	**mtDNA W**	**mtDNA total**	**Y D**	**Y W**	**Y total**
Wc	0.875-0.999	300	183	**51**	249		**6**	177	
BxW	0.638-0.875	38	23	**8**	30		**9**	14	
F1	0.443-0.513	8	5	3	5		4	1	
F2	0.416-0.587	2	2	1	1		1	1	
BxD	0.201-0.379	5	4	1	**4**		4	**0**	
Dc	0.002-0.139	138	101	138	**0**		101	**0**	
Total		491	318	202	289	491	125	193	318
Non-introgr.				139	279	418	105	191	298
Introgr.				**59**	**4**	**63**	**15**	**0**	**15**

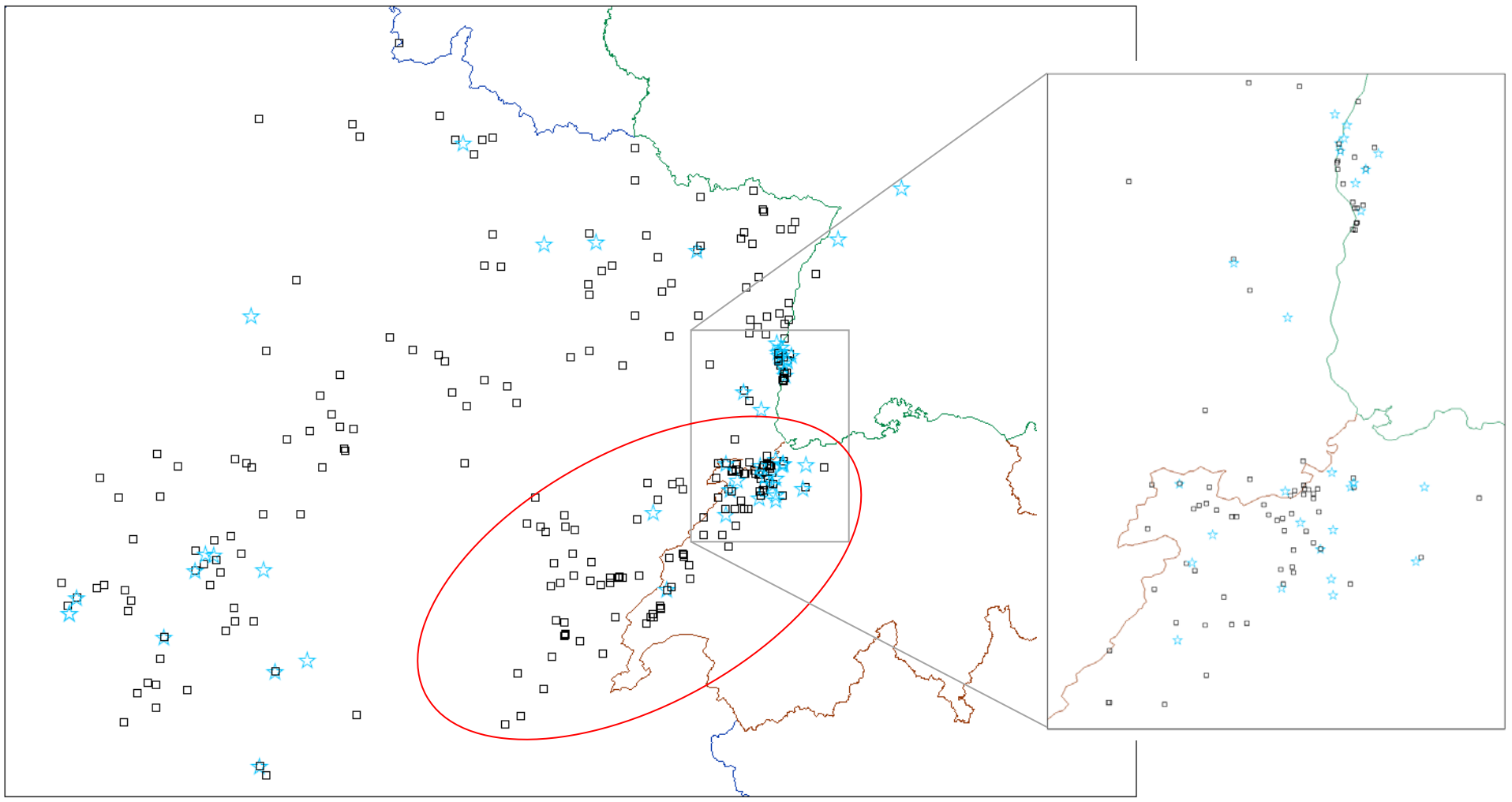

Figure 1: Map of wildcats (squares) and their hybrids (stars) around the Franco-Germano-Swiss borders (blue, green, and red lines respectively). The red oval defines the Franco-Swiss Jura population. For clarity, the inset shows an enlargement of an area with particularly dense sampling.

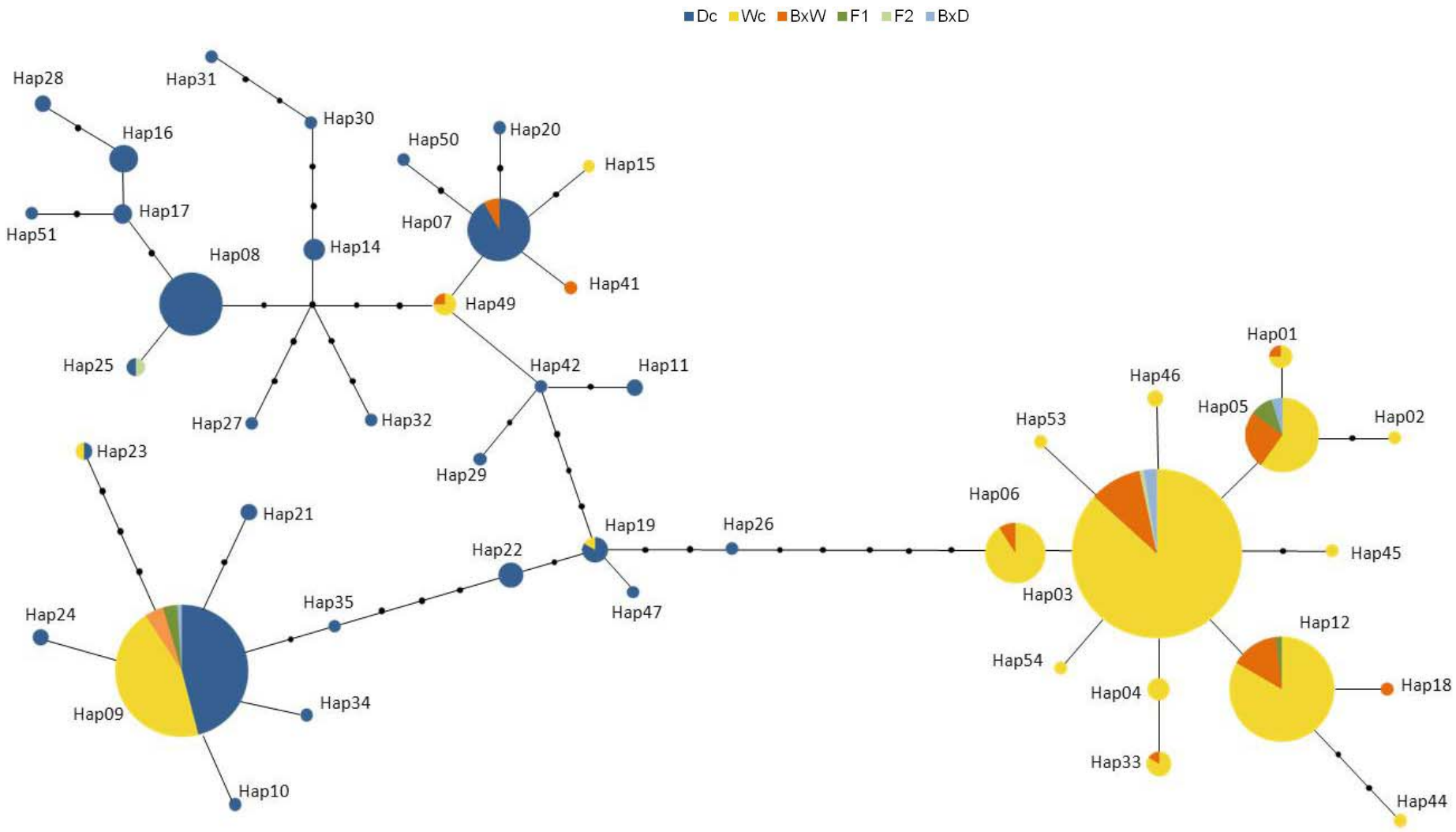

Figure 2: mtDNA haplotype network of wildcats, domestic cats, and hybrids. Colours correspond to the six genealogical categories defined through autosomal nuclear markers. Size of circles is proportional to the number of individuals observed. Each dot corresponds to one mutation.

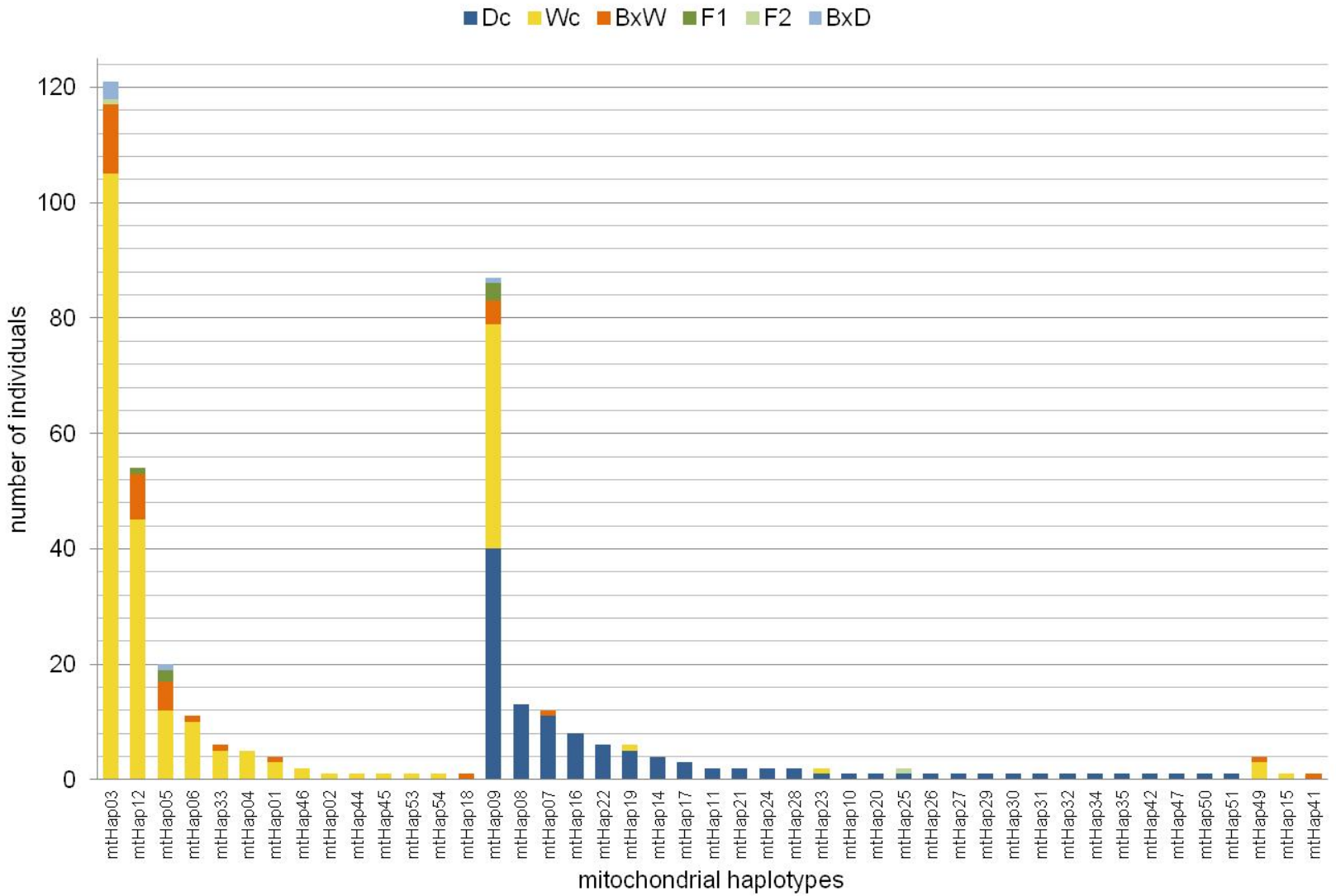

Figure 3: Frequency of mtDNA haplotypes in the six genealogical categories defined through autosomal nuclear markers.

Supporting Information 1: Results of genetic analyses of 491 cat samples. qWc = proportion of membership to wildcat cluster (Structure), SNP_6, resp. 8categ= Newhybrids genealogical categories: Wc = wildcat, BxW = backcross into wildcat, F1 = first generation hybrid, F2 = offspring of F1xF1, BxD = backcross into domestic cat, Dc = domestic cat, PostProb6c resp. 8c= posterior probability of assessed category, M = male, F= female, U= unclear, D = domestic, W = wild, mt_haplo = mitochondrial haplotype, gamek.= gamekeeper, NHM = Natural History Museum (Chd= Chaux-de-Fonds, Lau= Lausanne, Bas= Basel, Ber =Berne, Neu= Neuchâtel, Olte= Olten, Por= Porrentruy, Solo= Solothurn)

ID	Collection Name	SampleType	Source	Country	Longitude	Latitude	Sex	Year	qWc	SNP_6categ	PostProb6c	SNP8categ.	PostProb8c	SNP-Y	SNP-mtDNA	SMCY	mt_haplo
WK002	Saicourt (Saules)	blood	FIWI	CH	7.17844	47.24402	M	2009	0.997	Wc	1	Wc	1	W	W		NA
WK017	Beaumont	tissue	gamek.	F	5.84185	46.08114	F	2009	0.998	Wc	1	Wc	1	F	W		NA
WK018	Vicques	faeces	FIWI	CH	7.44459	47.34146		2009	0.963	Wc	1	Wc	0.97466	W	D		NA
WK019	Vuache	hair	Patry	F	5.91784	46.10442		2009	0.007	Dc	1	Dc	0.99998	D	D		NA
WK020	W09/2354	tissue	FIWI	CH	7.31942	47.40076	M	2005	0.981	Wc	1	Wc	0.99991	W	D		Hap09
WK022	-	tissue	FIWI	F	7.27110	47.47274	M	2006	0.892	Wc	0.89549	BxWxW	0.99969	W	D		Hap09
WK024	w05/3354	tissue	FIWI	CH	7.56194	47.44752	M	2005	0.693	BxW	1	BxW	0.99998	D	W		Hap05
WK026	W07/1779	tissue	FIWI	CH	7.03901	47.16627	M	2007	0.713	BxW	1	BxW	0.99991	W	W		NA
WK027	W07/3564	tissue	FIWI	CH	7.37914	47.29584	M	2007	0.998	Wc	1	Wc	0.99999	W	D		NA
WK028	W08/4868	tissue	FIWI	CH	7.45189	47.42803	M	2008	0.999	Wc	1	Wc	1	W	W		Hap33
WK033	W09/01563	tissue	FIWI	CH	7.15351	47.46118	F	2008	0.997	Wc	1	Wc	0.99999	F	W		Hap12
WK035	W09/01568	tissue	FIWI	CH	7.36251	47.36841	F	2009	0.918	Wc	0.99986	BxWxW	0.90969	W	W		NA
WK036	W09/01571	tissue	FIWI	CH	7.07560	47.31435	F	2009	0.989	Wc	1	Wc	0.99923	F	D		NA
WK037	W09/857/43=822	tissue	FIWI	CH	6.69148	46.78807	F	2009	0.999	Wc	1	Wc	1	F	W		Hap12
WK041	W09/2395	tissue	FIWI	CH	7.15598	47.36899	M	2009	0.869	BxW	0.92426	BxWxW	0.99731	W	W		NA
WK045	w09/3506	tissue	FIWI	CH	6.48617	46.73368	M	2008	0.698	BxW	0.99974	BxW	0.99688	W	W		NA
WK046	5100, w4	historic	Hertwig	D	10.73385	51.28639	M	1999	0.99	Wc	1	Wc	0.99998	W	W		Hap03
WK047	7920,w5	historic	Hertwig	D	11.01354	51.34370	M	2002	0.999	Wc	1	Wc	1	W	D		NA
WK049	ChdF Nr. 05031	historic	NHMChd	CH	6.84055	47.15146		2005	0.998	Wc	1	Wc	1	W	W		Hap05
WK050	21131, M37	historic	NHMLau	CH	6.36131	46.56458	M	1993	0.96	Wc	1	Wc	0.99831	W	W		Hap06
WK052	Basel Nachzügler 1991	tissue	NHMBas	CH	7.42670	47.44759	M	1991	0.996	Wc	1	Wc	1	W	W		Hap03
WK054	7165; 305/2005	historic	NHMBer	CH	7.49358	47.23720	M	2005	0.506	F1	1	F1	1	W	D		NA
WK055	9419	tissue	NHMNeu	CH	7.12055	47.42736		2006	0.992	Wc	1	Wc	0.99973	F	D		Hap09

WK056	33005	historic	NHMOlte	CH	7.75332	47.30055	F	2002	0.999	Wc	1	Wc	1	F	W		Hap12
WK058	N°: 756.0981 0045 9791	blood	Ecoffey	CH	6.48539	46.72745	M	2010	0.999	Wc	1	Wc	1	W	W		Hap44
WK059	7928, w29	historic	Hertwig	D	10.29832	50.89395	F	2002	0.988	Wc	1	Wc	0.99995	F	D		NA
WK060	5098, w31	historic	Hertwig	D	10.51110	51.10864	F	1996	0.996	Wc	1	Wc	0.99999	F	D		NA
WK061	5097, w32	historic	Hertwig	D	10.87105	51.36919	F	1996	0.787	BxW	1	BxW	0.57738	F	D		NA
WK062	5102, w34	historic	Hertwig	D	11.01354	51.34370	F	1997	0.855	BxW	0.99406	BxWxW	0.98165	F	D		NA
WK063	Strasse Ziefen	hair	Sutter	CH	7.70371	47.42750		2010	0.009	Dc	1	Dc	0.99991	F	D		Hap24
WK068	W10/3858	tissue	FIWI	CH	7.38180	47.28505	F	2010	0.998	Wc	1	Wc	1	F	W		Hap03
WK070	Monnerat1	tissue	Monnerat	CH	7.06223	47.47486		2006	0.007	Dc	1	Dc	0.99996	D	D		NA
WK074	GE 1631.05	historic	NHMGen	F				1980	0.998	Wc	1	Wc	1	W	W		Hap33
WK075	Nr. 03043	historic	NHMChd	CH	6.51962	46.92041		1999	0.998	Wc	1	Wc	1	W	W		Hap12
WK076	Monnerat4	historic	Monnerat	CH	7.09311	47.31620		2005	0.831	BxW	0.99999	BxWxW	0.96077	W	W	W	Hap03
WK077	Monnerat2	tissue	Monnerat	CH	7.10403	47.42057	M	2006	0.998	Wc	1	Wc	1	W	D		Hap09
WK078	CO403/Mam	historic	NHMPor	CH	7.35917	47.39180		1992	0.999	Wc	1	Wc	1	W	W		Hap12
WK079	Nr. 620/94.3137A	historic	NHMNeu	CH	6.64307	46.93977	M	2002	0.994	Wc	1	Wc	0.99996	W	W		Hap12
WK080	Nr. 536/94.3028	historic	NHMNeu	CH	6.65076	46.93038		1992	0.98	Wc	1	Wc	0.99798	W	W		NA
WK081	64/90, 1031117	historic	NHMBer	CH	6.42065	46.63439		1990	0.999	Wc	1	Wc	1	W	W		NA
WK082	1031114	historic	NHMBer	CH	7.24006	47.19018		1989	0.999	Wc	1	Wc	1	W	D	W	Hap09
WK084	Nr. 21951	historic	NHMLau	CH	6.69103	46.86768		1935	0.997	Wc	1	Wc	0.99998	W	W		Hap12
WK085	Nr. 29397	historic	NHMLau	CH	6.35566	46.58611		1941	0.997	Wc	1	Wc	1	W	W		Hap05
WK088	W12/602	tissue	FIWI	CH	7.34616	47.25687	M	2012	0.8	BxW	1	BxWxW	0.513	W	D		NA
WK090	FS2101	tissue	ONCFS	F	5,39280	47,13354	F	1998	0.914	Wc	0.99995	BxWxW	0.88613	F	W		NA
WK091	FS2103	tissue	ONCFS	F	4,73269	47,52830	M	2002	0.997	Wc	1	Wc	1	W	D		NA
WK092	FS2104	tissue	ONCFS	F	4,63729	47,95518	F	2001	0.989	Wc	1	Wc	0.99937	F	W		Hap04
WK093	FS21Tr	tissue	ONCFS	F	5,27542	47,15658	F	2004	0.997	Wc	1	Wc	1	F	W		Hap12
WK094	FS2502	tissue	ONCFS	F	6,43719	47,27482	M	2003	0.999	Wc	1	Wc	1	W	W		Hap12
WK095	FS2503	tissue	ONCFS	F	6,35251	47,37441	F	2006	0.997	Wc	1	Wc	0.99998	F	W		Hap01
WK096	FS2505	tissue	ONCFS	F	6,39612	47,19744	M?	2005	0.867	BxW	0.59109	BxWxW	0.99877	W	W		NA
WK097	FS2506	tissue	ONCFS	F	6,64560	47,37321	M	2005	0.978	Wc	1	Wc	0.99993	W	W		Hap06
WK098	FS2507	tissue	ONCFS	F	6,24848	47,15955	M	2005	0.973	Wc	1	Wc	0.9868	W	W		Hap12

WK099	FS2508	tissue	ONCFS	F	6,66476	47,32885	M	2005	0.998	Wc	1	Wc	1	W	W	Hap12
WK100	FS2509	tissue	ONCFS	F	6,58089	47,36460	F	2005	0.999	Wc	1	Wc	1	F	D	NA
WK101	FS3909	tissue	ONCFS	F	5,58287	46,48050	M	2002	0.999	Wc	1	Wc	1	W	W	Hap12
WK102	FS3912	tissue	ONCFS	F	6,07171	46,81864	M	2003	0.997	Wc	1	Wc	0.99999	W	W	Hap05
WK103	FS3914	tissue	ONCFS	F	5,82132	46,80031	M	2003	0.999	Wc	1	Wc	1	W	W	Hap03
WK104	FS3915	tissue	ONCFS	F	5,46784	46,77492	F	2003	0.995	Wc	1	Wc	0.99996	F	W	Hap12
WK105	FS3918	tissue	ONCFS	F	5,58214	46,55712	M	2003	0.998	Wc	1	Wc	1	W	W	Hap06
WK106	FS3919	tissue	ONCFS	F	5,98949	46,78313	F	2004	0.965	Wc	1	Wc	0.9893	F	W	Hap12
WK107	FS3920	tissue	ONCFS	F	5,50226	46,91482	M	2004	0.996	Wc	1	Wc	0.99999	W	W	Hap12
WK108	FS3921	tissue	ONCFS	F	5,99430	46,81619	F	2004	0.993	Wc	1	Wc	0.99976	F	W	Hap03
WK109	FS3923	tissue	ONCFS	F	6,09824	46,80975	F	2004	0.968	Wc	1	Wc	0.99217	F	W	Hap06
WK110	FS3924	tissue	ONCFS	F	5,82134	46,80032	F	2003	0.999	Wc	1	Wc	1	F	D	NA
WK111	FS39B	tissue	ONCFS	F	5,90494	46,77004	F	2005	0.998	Wc	1	Wc	1	F	W	Hap03
WK112	FS39C	tissue	ONCFS	F	5,43618	47,10541	M	2001	0.995	Wc	1	Wc	0.99994	W	W	Hap12
WK113	FS39D	tissue	ONCFS	F	6,06792	46,81381	F	2005	0.965	Wc	1	Wc	0.99685	F	W	Hap12
WK114	FS39E	tissue	ONCFS	F	5,55255	46,79453	F	2005	0.997	Wc	1	Wc	0.99999	F	W	Hap12
WK115	FS39F	tissue	ONCFS	F	5,50662	46,56952	M ?	2005	0.992	Wc	1	Wc	0.99992	W	W	Hap03
WK116	FS39G	tissue	ONCFS	F	5,67779	46,83750	M	2004	0.998	Wc	1	Wc	1	W	W	Hap12
WK117	FS39H	tissue	ONCFS	F	5,66922	46,96888	F	2004	0.998	Wc	1	Wc	1	F	W	Hap12
WK118	FS39I	tissue	ONCFS	F	6,02766	46,57196	M	2005	0.995	Wc	1	Wc	0.99999	W	W	Hap12
WK119	FS39J	tissue	ONCFS	F	5,90260	46,35676	M	2005	0.998	Wc	1	Wc	1	W	W	Hap06
WK120	FS39K	tissue	ONCFS	F	5,71053	46,43767	F	2006	0.998	Wc	1	Wc	1	F	W	Hap06
WK121	FS39L	tissue	ONCFS	F	5,46009	46,34803	M	2006	0.998	Wc	1	Wc	1	W	W	Hap03
WK122	FS39M	tissue	ONCFS	F	5,83657	46,91516	M	2006	0.995	Wc	1	Wc	0.99995	W	D	Hap09
WK123	FS39N	tissue	ONCFS	F	5,69906	47,11191	M	2005	0.995	Wc	1	Wc	0.99998	W	W	Hap03
WK124	FS39O	tissue	ONCFS	F	5,58506	46,48457	F	2005	0.996	Wc	1	Wc	0.99999	F	W	Hap12
WK140	1031075, 664/69	historic	NHMBer	CH	7.16965	47.41580	F	1969	0.992	Wc	1	Wc	0.99997	W	W	NA
WK142	1056672	historic	NHMBer	CH	7.55796	47.44573	M	2007	0.006	Dc	1	Dc	0.99997	D	D	Hap08
WK145	MtDar	blood	gamek.	CH	6.83113	47.04964	F	2012	0.905	Wc	0.99988	BxWxW	0.92086	F	W	Hap45
WK147	CO344/M, BNM: 12537	historic	NHMPor	CH	7.14082	47.42949	F	1988	0.999	Wc	1	Wc	1	F	D	NA
WK148	Asuel, M16	historic	NHMPor	CH	7.21344	47.40060		1973	0.998	Wc	1	Wc	1	W	D	Hap09
WK149	Le Locle 37.01.1059	historic	NHMChd	CH	6.69529	47.03413		1935	0.002	Dc	1	Dc	1	D	D	Hap42
WK152	Balmberg, 1598-1915	historic	NHMSolo	CH	7.54433	47.25686		1915	0.997	Wc	1	Wc	0.99999	W	W	Hap03

WK154	1031076	historic	NHMBer	CH	7.30744	47.42098		1970	0.997	Wc	1	Wc	0.99999	W	W	Hap33
WK155	1031097, 71/1976	historic	NHMBer	CH	7.34394	47.38954	M	1976	0.999	Wc	1	Wc	1	W	W	Hap12
WK156	1031098, 287/76	historic	NHMBer	CH	6.97880	47.26634	M	1976	0.999	Wc	1	Wc	1	W	W	Hap03
WK157	1031104, 305/79	historic	NHMBer	CH	7.11529	47.09275	M	1979	0.999	Wc	1	Wc	1	W	W	Hap03
WK158	10106	historic	NHMSolo	CH	7.49083	47.26881	M	1981	0.873	BxW	0.50367	BxWxW	0.99904	F	D	Hap41
WK159	1031109, 1/83	historic	NHMBer	CH	7.19586	47.18964	M	1983	0.999	Wc	1	Wc	1	W	W	NA
WK160	Rocourt	historic	NHMPor	CH	6.96850	47.38637		1984	0.999	Wc	1	Wc	1	F	W	NA
WK161	1031112, 375/1984	historic	NHMBer	CH	7.09884	47.29868	M	1984	0.999	Wc	1	Wc	1	W	W	Hap03
WK162	Nr 428/94.1696	historic	NHMNeu	CH	6.65090	46.92139	M	1984	0.999	Wc	1	Wc	1	W	W	Hap03
WK166	1050030, 369/00	historic	NHMBer	CH	7.35143	47.26362	M	2000	0.999	Wc	1	Wc	1	W	W	Hap12
WK167	1047416, 458/97	historic	NHMBer	F			M	1991	0.998	Wc	1	Wc	1	W	W	Hap03
WK168	tag_1137 D-WK282	blood	FVA	D	7.61007	47.95070	F	2010	0.944	Wc	1	Wc	0.98219	F	W	NA
WK169	tag_1138 D-WK283	blood	FVA	D	7.62254	47.96250	M	2010	0.968	Wc	1	Wc	0.98847	W	W	NA
WK170	tag_1139 D-WK324	blood	FVA	D	7.61915	47.99190	F	2010	0.994	Wc	1	Wc	0.99998	F	W	NA
WK171	tag_1140, ID_K_036 D-WK325	blood	FVA	D	7.63927	47.98680	M	2010	0.638	BxW	0.99984	BxW	0.99962	D	W	Hap05
WK172	tag_1141 D-WK326	blood	FVA	D	7.61915	47.99190	F	2010	0.996	Wc	1	Wc	0.99998	F	W	NA
WK173	tag_1238 D-WK327	blood	FVA	D	7.57798	48.08680	F	2010	0.999	Wc	1	Wc	1	F	W	NA
WK174	tag_1239 D-WK328	blood	FVA	D	7.58876	48.10560	F	2010	0.999	Wc	1	Wc	1	F	W	NA
WK175	tag_1236, ID_K_043 D-WK365	blood	FVA	D	7.68770	48.10840	M	2010	0.882	Wc	0.74783	BxWxW	0.99862	D	W	Hap03
WK176	tag_1237, ID_K_040 D-WK366	blood	FVA	D	7.59114	48.04050	M	2010	0.999	Wc	1	Wc	1	W	W	Hap03
WK177	tag_1240 D-WK367	blood	FVA	D	7.57798	48.08680	F	2010	0.998	Wc	1	Wc	1	F	W	Hap03
WK178	tag_1551, ID_K_038 D-WK504	blood	FVA	D	7.61619	48.00490	M	2011	0.996	Wc	1	Wc	0.99999	W	W	Hap03
WK179	tag_1585 D-WK505	blood	FVA	D	7.57822	48.08640	F	2011	0.999	Wc	1	Wc	1	F	W	Hap03
WK180	tag_1582 D-WK506	blood	FVA	D	7.58880	48.10690	F	2011	0.647	BxW	0.99748	BxW	0.98926	F	W	Hap12
WK181	tag_1712 D-WK627	blood	FVA	D	7.58620	48.12170	M	2012	0.462	F1	1	F1	1	D	W	Hap05
WK182	tag_1554 D-WK628	blood	FVA	D	7.64813	48.20020	M	2012	0.998	Wc	1	Wc	1	W	W	Hap01
WK183	tag_2063 D-WK658	blood	FVA	D	7.66015	48.06800	M	2012	0.505	F1	0.99694	F1	0.99468	U	W	Hap05
WK184	tag_2061, ID_K_053 D-WK660	blood	FVA	D	7.70055	48.09740	F	2012	0.736	BxW	1	BxW	0.99684	F	W	NA
WK185	tag_1583 D-WK689	blood	FVA	D	7.57672	48.08310	M	2012	0.999	Wc	1	Wc	1	W	W	Hap05
WK186	tag_2060 D-WK691	blood	FVA	D	7.66015	48.06800	M	2012	0.937	Wc	1	Wc	0.66352	D	W	Hap03

WK187	ID_K_001 D-WK168	tissue	FVA	D	7.64427	47.99750	M	2006	0.998	Wc	1	Wc	1	W	W		Hap05
WK189	ID_K_021 D-WK271	tissue	FVA	D	7.62765	48.04210	M	2009	0.749	BxW	1	BxW	0.98692	D	W		Hap12
WK190	ID_K_029 D-WK348	tissue	FVA	D	8.82415	49.05360	M	2010	0.002	Dc	1	Dc	1	D	D		NA
WK191	ID_K_037 D-WK565	tissue	FVA	D	7.96884	48.58020	M	2011	0.937	Wc	1	Wc	0.68519	W	W		NA
WK192	ID_K_059 D-WK625	tissue	FVA	D	8.19197	48.78190	M	2011	0.443	F1	1	F1	1	D	W		NA
WK193	ID_K_050 D-WK690	tissue	FVA	D	7.61244	48.15630	F	2012	0.74	BxW	0.99999	BxW	0.94735	F	W		Hap05
WK194	ID_K_032 D-WK422	tissue	FVA	D	8.13246	48.30290		2010	0.006	Dc	1	Dc	0.99998	D	D		NA
WK196	ID_K_042 D-WK611	tissue	FVA	D	7.61910	47.67460		2011	0.017	Dc	1	Dc	0.99953	D	D		NA
WK197	W12/4694	tissue	FIWI	CH	7.51025	47.47756	F	2012	0.825	BxW	0.99999	BxWxW	0.94471	F	W		Hap12
WK198		tissue	FVA	D	9.88129	48.06630	M	2011	0.015	Dc	1	Dc	0.9996	D	D		NA
WK199	ID_K_057	tissue	FVA	D	7.77771	48.03600	M	2011	0.009	Dc	1	Dc	0.99993	D	D		NA
WK200	N° 2121116-017	tissue	Boujon	CH	7.04238	46.96825	M	2012	0.931	Wc	1	BxWxW	0.62504	W	W		Hap03
WK201	W12/5196	tissue	FIWI	CH	7.73886	47.29440	M	2012	0.416	F2	0.99341	F2	0.99358	W	D		Hap25
WK202	W12/5238	tissue	FIWI	CH	7.03717	47.19940	M	2012	0.999	Wc	1	Wc	1	W	W		Hap03
WK203	FS0301	tissue	ONCFS	F	2,86159	46,58712	M	2001	0.917	Wc	0.99972	BxWxW	0.9082	W	W	W	Hap03
WK204	FS0317	tissue	ONCFS	F	2,69042	46,67343	M	1994	0.944	Wc	1	Wc	0.91628	W	W		Hap12
WK205	FS1813	tissue	ONCFS	F	2,34265	47,01111	F	1994	0.954	Wc	1	Wc	0.91381	F	D		Hap09
WK206	FS21	tissue	ONCFS	F	5,35234	47,31302		2003	0.998	Wc	1	Wc	1	W	W		Hap03
WK207	FS2105	tissue	ONCFS	F	4,76223	47,87441	F	2002	0.949	Wc	1	Wc	0.87693	F	W		NA
WK208	FS2510	tissue	ONCFS	F	6,24551	46,81886	M	2005	0.987	Wc	1	Wc	0.99953	W	W		NA
WK209	FS39A	tissue	ONCFS	F	5,61459	47,13365	M	2004	0.999	Wc	1	Wc	1	W	W		NA
WK210	FS45C	tissue	ONCFS	F	2,83938	47,51845	F	2005	0.989	Wc	1	Wc	0.99999	F	W		NA
WK211	FS6701	tissue	ONCFS	F	7,69872	48,31131	M	1994	0.998	Wc	1	Wc	1	W	W		NA
WK212	FS6702	tissue	ONCFS	F	7,65747	48,28850	M	1996	0.996	Wc	1	Wc	0.99993	W	W		NA
WK213	FS6705	tissue	ONCFS	F	7,40462	48,78594	F	1997	0.998	Wc	1	Wc	1	F	W		NA
WK214	FS6706	tissue	ONCFS	F	7,49978	48,34272	F	1997	0.948	Wc	1	Wc	0.96513	F	W		NA
WK215	FS6708	tissue	ONCFS	F	7,80052	48,89891	M	1997	0.897	Wc	0.9996	BxWxW	0.96541	W	W	-	NA
WK216	FS6709	tissue	ONCFS	F	7,70455	48,41119	M	1997	0.998	Wc	1	Wc	1	W	W		Hap01
WK217	FS6712	tissue	ONCFS	F	7,47912	48,23699	F	1999	0.894	Wc	0.99352	BxWxW	0.99737	F	W		Hap03
WK218	FS6716	tissue	ONCFS	F	7,66559	48,86183	M	2001	0.998	Wc	1	Wc	1	W	W		Hap03
WK219	FS6717	tissue	ONCFS	F	7,41160	48,27831	M	2002	0.999	Wc	1	Wc	1	W	W		Hap02
WK220	FS6719	tissue	ONCFS	F	7,34464	48,32789	M	2002	0.995	Wc	1	Wc	0.99998	W	W		Hap04
WK221	FS6720	tissue	ONCFS	F	7,33098	48,24942	F	2002	0.999	Wc	1	Wc	1	F	D		Hap09
WK222	FS6721	tissue	ONCFS	F	7,44629	48,58309	M	2003	0.994	Wc	1	Wc	0.99995	W	W		NA
WK223	FS6730	tissue	ONCFS	F	7,57877	48,17941	M	2004	0.846	BxW	0.9986	BxWxW	0.99336	W	W	W	Hap05

WK224	FS67A	tissue	ONCFS	F	7,49685	48,54783	M	2005	0.008	Dc	1	Dc	0.99999	D	D		NA
WK225	FS67C	tissue	ONCFS	F	7,33561	48,85629	M	2005	0.909	Wc	0.99923	BxWxW	0.98583	W	W	W	NA
WK226	FS67H	tissue	ONCFS	F	7,32227	48,52147		2006	0.985	Wc	1	Wc	0.99979	F	W		NA
WK227	FS6807	tissue	ONCFS	F	7,25894	47,89897	M	2000	0.753	BxW	1	BxW	0.9719	W	W	W	Hap12
WK228	FS6810	tissue	ONCFS	F	7,40829	47,78687	F	2001	0.7	BxW	0.99979	BxW	0.99764	F	W		Hap03
WK229	FS6811	tissue	ONCFS	F			F	2002	0.987	Wc	1	Wc	0.99981	F	W		NA
WK230	FS6812	tissue	ONCFS	F			M	avant 2002	0.369	BxD	0.87329	BxD	0.98105	D	W	D	Hap03
WK231	FS6814	tissue	ONCFS	F	7,25833	47,90642	M	2003	0.983	Wc	1	Wc	0.99909	W	W		NA
WK232	FS68A	tissue	ONCFS	F	7,30057	47,84268	M	2004	0.998	Wc	1	Wc	1	W	W		NA
WK237	W13_6341	tissue	FIWI	CH	6.52826	46.74421	F	2013	0.999	Wc	1	Wc	1	F	W		NA
WK238	W13_6389	tissue	FIWI	CH	6.42759	46.62310	F	2013	0.996	Wc	1	Wc	0.99998	F	W		NA
WK245	Oltingue	hair	Weber	F	7,42801	47,50290		2006	0.978	Wc	1	Wc	0.99948	F	D		NA
WK247	ID_K_041	tissue	FVA	D	7.62858	48.09240		2011	0.974	Wc	1	Wc	0.99948	W	D	-	NA
WK248	ID_K_048	tissue	FVA	D	7.74670	48.19020		2011	0.015	Dc	1	Dc	0.9998	D	D		NA
WK249	Hänsel	hair	FVA	D				2009	0.92	Wc	0.99997	BxWxW	0.86003	W	W	-	NA
WK250	Gretel	hair	FVA	D				2009	0.999	Wc	1	Wc	1	F	W		NA
WK251	tag_1554	hair	FVA	D	7.62358	47.96370		2011	0.97	Wc	1	Wc	0.9994	F	W		NA
WK252	tag_1553	hair	FVA	D	7.60128	48.13080		2011	0.759	BxW	1	BxW	0.77821	F	W		Hap01
WK253	KS01	hair	FVA	D	7.66015	48.06800		2012	0.507	F1	1	F1	1	D	W	D	NA
WK254	BS02	hair	FVA	D	7.62675	47.99160		2012	0.978	Wc	1	Wc	0.99766	W	W		NA
WK255	Phoebe	hair	FVA	D	8.80144	49.06440		2012	0.854	BxW	0.99838	BxWxW	0.99182	F	W		Hap03
WK256	BN03	blood	FVA	D	7.57694	48.06960		2012	0.997	Wc	1	Wc	0.99998	W	W		NA
WK257	D-WK614	hair	FVA	D	7.84822	47.98310		2011	0.139	Dc	0.99212	Dc	0.60966	D	D		NA
WK258	HH_05	hair	FVA	D	7.61765	47.94910		2013	0.96	Wc	1	Wc	0.98902	W	W		NA
WK259	JS06	hair	FVA	D	7.58448	48.11960		2013	0.908	Wc	0.99803	BxWxW	0.99449	W	W	D	NA
WK260	W13/6475	tissue	FIWI	CH	6.42900	46.61405	F	2013	0.999	Wc	1	Wc	1	F	W		NA
WK261	W13/6478	tissue	FIWI	CH	7.11997	47.19165	M	2013	0.999	Wc	1	Wc	1	W	W		NA
WK262	W13/6671	tissue	FIWI	CH	7.77601	47.43851	F	2013	0.875	BxW	0.86115	BxWxW	0.99866	F	W		Hap05
WK263	Zeglingen	tissue	FIWI	CH	7.93085	47.41011	M	2013	0.997	Wc	1	Wc	0.99999	W	D	W	Hap09
WK264	APJ0510	tissue	ONCFS	F	5,03198	49,43650	F	2004	0.02	Dc	1	Dc	0.99922	F	D	-	Hap07
WK265	APJ0511	tissue	ONCFS	F	5,57132	46,47243	M	2002	0.995	Wc	1	Wc	0.99996	W	W		Hap46
WK266	APJ0512	tissue	ONCFS	F			M	2003	0.005	Dc	1	Dc	1	D	D		Hap47
WK267	APJ0513	tissue	ONCFS	F	5,22540	46,13476		2005	0.007	Dc	1	Dc	0.99999	D	D		Hap16
WK268	APJ052	tissue	ONCFS	F	4,79511	49,45884	M	2004	0.011	Dc	1	Dc	0.99993	D	D		Hap07

WK269	APJ053	tissue	ONCFS	F	7,35863	48,68926	F	2004	0.003	Dc	1	Dc	1	F	D	Hap16
WK270	APJ056	tissue	ONCFS	F	5,05955	49,48395	F	2004	0.999	Wc	1	Wc	1	F	W	Hap05
WK271	APJ057	tissue	ONCFS	F	4,80268	49,41400	M	2004	0.01	Dc	1	Dc	0.99994	D	D	Hap09
WK273	FS0104	tissue	ONCFS	F	5,17646	46,00042	F	1998	0.959	Wc	1	Wc	0.99238	F	W	Hap46
WK274	FS0108	tissue	ONCFS	F			F	2003	0.447	F1	1	F1	1	F	W	Hap12
WK275	FS01X1	tissue	ONCFS	F	5,37382	46,15531	F	1999	0.997	Wc	1	Wc	0.99998	F	W	Hap12
WK276	FS01X2	tissue	ONCFS	F	5,17647	46,00043	F	1998	0.999	Wc	1	Wc	1	F	W	Hap12
WK277	FS01X3	tissue	ONCFS	F	5,03955	45,95306	M	env 1995	0.994	Wc	1	Wc	0.99999	W	W	Hap06
WK278	FS0303	tissue	ONCFS	F	3,33300	46,35593		1997	0.379	BxD	0.79842	BxD	0.96533	D	W	D	Hap03
WK280	FS0313	tissue	ONCFS	F	2,69268	46,59035	F	1999	0.948	Wc	1	Wc	0.81798	F	W		Hap03
WK282	FS0316	tissue	ONCFS	F	2,69042	46,67343	F	2001	0.928	Wc	1	Wc	0.72412	F	W		Hap03
WK283	FS03X1	tissue	ONCFS	F	3,75309	46,02162	M	1999	0.916	Wc	0.99982	BxWxW	0.99101	U	W	D	Hap12
WK285	FS0803	tissue	ONCFS	F	4,79513	49,45885	M	1996	0.803	BxW	1	BxW	0.6145	W	W	-	Hap03
WK286	FS0804	tissue	ONCFS	F	4,57262	49,62425	F	1998	0.994	Wc	1	Wc	0.99997	F	D		Hap09
WK287	FS0805	tissue	ONCFS	F	4,71402	49,47846	F	2003	0.947	Wc	1	Wc	0.88962	F	D	-	Hap09
WK288	FS08A	tissue	ONCFS	F	4,96907	49,47281	F	2005	0.981	Wc	1	Wc	0.99852	F	W		NA
WK289	FS08B	tissue	ONCFS	F	4,88327	49,38505	M	2005	0.876	Wc	0.85938	BxWxW	0.99975	W	W	-	Hap03
WK290	FS08C	tissue	ONCFS	F	4,80270	49,41401	F	2005	0.002	Dc	1	Dc	1	F	D		Hap08
WK291	FS08D	tissue	ONCFS	F	4,85729	49,41394	M	2005	0.009	Dc	1	Dc	0.99992	D	D		Hap08
WK292	FS08E	tissue	ONCFS	F	4,81187	49,43582	F	2005	0.006	Dc	1	Dc	0.99999	F	D		Hap09
WK294	FS1002	tissue	ONCFS	F	4,28931	48,21860	M	1997	0.994	Wc	1	Wc	0.99995	W	W		Hap03
WK295	FS1004	tissue	ONCFS	F	4,08255	48,29576	M	1997	0.994	Wc	1	Wc	1	W	D	-	Hap09
WK296	FS1010	tissue	ONCFS	F	4,57971	48,14804	M	1996	0.996	Wc	1	Wc	0.99998	W	W		Hap03
WK297	FS1012	tissue	ONCFS	F	4,52344	48,18727	M	1997	0.997	Wc	1	Wc	1	W	D	-	Hap09
WK298	FS1501	tissue	ONCFS	F	3,15827	45,21432	M	2004	0.929	Wc	1	BxWxW	0.50831	W	W	W	Hap03
WK299	FS1802	tissue	ONCFS	F	2,56773	46,88407	F	1991	0.901	Wc	0.9968	BxWxW	0.99818	F	D		Hap09
WK301	FS1804	tissue	ONCFS	F	2,65891	47,10090	M	1994	0.904	Wc	0.99949	BxWxW	0.9784	W	W	-	Hap03
WK302	FS1805	tissue	ONCFS	F	2,50641	47,08035	M	1994	0.981	Wc	1	Wc	0.99991	W	W		Hap03
WK303	FS1806	tissue	ONCFS	F	2,41547	46,93066	F	1991	0.99	Wc	1	Wc	0.99938	F	W		Hap03
WK304	FS1809	tissue	ONCFS	F	2,94650	46,90582	M	1993	0.866	BxW	0.90952	BxWxW	0.99821	W	D	-	Hap09
WK305	FS1811	tissue	ONCFS	F	2,74731	46,99949	F	1989	0.984	Wc	1	Wc	0.99965	F	W		Hap03
WK307	FS1824	tissue	ONCFS	F	2,47474	46,80861	M	1995	0.959	Wc	1	Wc	0.99438	W	W		Hap06
WK308	FS1826	tissue	ONCFS	F	2,74730	46,99950	M	env 1996	0.875	Wc	0.86014	BxWxW	0.99948	W	D	-	Hap09

WK310	FS1830		tissue	ONCFS	F	2,50739	46,99230	F	2003	0.849	BxW	0.99027	BxWxW	0.99838	F	D		Hap09
WK311	FS1831		tissue	ONCFS	F	2,93732	47,23378	M	2001	0.988	Wc	1	Wc	0.99949	W	W		Hap03
WK312	FS1832		tissue	ONCFS	F	2,43843	46,99700	M	2002	0.513	F1	1	F1	1	D	D	D	Hap09
WK313	FS1833		tissue	ONCFS	F	2,35086	46,89509		2001	0.587	F2	1	F2	1	D	W	D	Hap03
WK314	FS1835		tissue	ONCFS	F	2,53313	46,95878	M	2001	0.982	Wc	1	Wc	0.99773	W	W		Hap12
WK315	FS1902		tissue	ONCFS	F	1,91032	45,63494	F	2004	0.008	Dc	1	Dc	0.9999	F	D		Hap07
WK316	FS2306		tissue	ONCFS	F	1,93747	46,21486	M	2001	0.988	Wc	1	Wc	0.99951	W	W		Hap03
WK317	FS2307		hair	ONCFS	F	2,01016	46,20348	M	2000	0.973	Wc	1	Wc	0.99905	W	W		Hap03
WK318	FS2310		tissue	ONCFS	F	2,01405	46,06388	F	2003	0.992	Wc	1	Wc	0.99982	F	W		Hap03
WK319	FS23C		tissue	ONCFS	F	1,74173	45,97580	M	2005	0.957	Wc	1	Wc	0.96636	W	W		Hap03
WK320	FS2501		tissue	ONCFS	F			F	avant 2002	0.997	Wc	1	Wc	1	F	W	-	Hap03
WK321	FS3601		tissue	ONCFS	F	1,25019	46,62367	F	1995	0.749	BxW	1	BxW	0.99601	F	W		Hap03
WK322	FS3603		tissue	ONCFS	F	1,72799	46,76866	M	1992	0.996	Wc	1	Wc	0.99999	W	W		Hap03
WK323	FS3604		tissue	ONCFS	F	1,31200	46,72162	M	1991	0.906	Wc	1	BxWxW	0.61787	W	D	-	Hap09
WK324	FS3605		tissue	ONCFS	F	1,17183	46,80976	M	1996	0.984	Wc	1	Wc	0.99856	W	D	-	Hap09
WK326	FS3634		tissue	ONCFS	F	1,75570	46,64276	M	2000	0.988	Wc	1	Wc	0.99874	W	D	-	Hap09
WK327	FS3635		tissue	ONCFS	F	1,23110	46,66966	F	2001	0.99	Wc	1	Wc	0.99985	F	D		Hap09
WK328	FS3638		tissue	ONCFS	F	1,79374	47,07581	F	2001	0.998	Wc	1	Wc	1	F	W		Hap03
WK330	FS3640		tissue	ONCFS	F				avant 2004	0.011	Dc	1	Dc	0.99979	F	D		Hap09
WK331	FS3641		tissue	ONCFS	F	2,07946	46,49190	M	2002	0.955	Wc	1	Wc	0.889	F	D	-	Hap09
WK332	FS3643		tissue	ONCFS	F	1,23110	46,66966	M	2003	0.998	Wc	1	Wc	1	W	D	W	Hap09
WK333	FS3644		tissue	ONCFS	F	1,31200	46,72162	F	2004	0.828	BxW	1	BxWxW	0.90907	F	D		Hap09
WK334	FS3645		tissue	ONCFS	F	1,25018	46,62367	M	2004	0.645	BxW	0.99913	BxW	0.99706	D	W	-	Hap03
WK335	FS3646		tissue	ONCFS	F	1,23110	46,66966	F	2001	0.964	Wc	1	Wc	0.99601	F	D		Hap09
WK336	FS36A		tissue	ONCFS	F	1,76543	47,03626	F	2004	0.004	Dc	1	Dc	0.99999	F	D		Hap08
WK337	FS39X1		tissue	ONCFS	F			F	env 1995	0.998	Wc	1	Wc	1	F	W		Hap03
WK339	FS4107		tissue	ONCFS	F	1,99548	47,59516	F	1998	0.995	Wc	1	Wc	0.99997	W	W	-	Hap03
WK340	FS4108		tissue	ONCFS	F	1,65626	47,32637	M	2002	0.976	Wc	1	Wc	0.99971	W	W		Hap03
WK341	FS41A		tissue	ONCFS	F	2,06192	47,53041	M	2006	0.006	Dc	1	Dc	0.99994	D	D		Hap09
WK342	FS41B		tissue	ONCFS	F	1,64819	47,36892	F	2004	0.072	Dc	0.99992	Dc	0.90095	F	D		Hap08
WK343	FS41C		tissue	ONCFS	F	2,02559	47,34064	F	2004	0.978	Wc	1	Wc	0.99814	F	W		Hap03
WK344	FS4504		tissue	ONCFS	F	2,79410	47,54301	F	2000	0.987	Wc	1	Wc	0.99938	F	W		Hap03

WK345	FS4505	tissue	ONCFS	F	2,79412	47,54302	F	2000	0.991	Wc	1	Wc	0.99998	F	W		Hap12
WK346	FS4506	tissue	ONCFS	F	2,48805	47,80013	M	2003	0.036	Dc	0.99997	Dc	0.98916	D	D		Hap50
WK347	FS4507	tissue	ONCFS	F	2,69173	47,56840	M	2002	0.999	Wc	1	Wc	1	W	W		Hap03
WK348	FS4508	tissue	ONCFS	F	1,98990	48,02473	M	2003	0.002	Dc	1	Dc	1	D	D		Hap51
WK349	FS4509	tissue	ONCFS	F	2,79416	47,54305	M	2004	0.997	Wc	1	Wc	1	W	W		Hap03
WK353	FS5102	tissue	ONCFS	F	4,95521	48,71497	M	1996	0.961	Wc	1	Wc	0.98706	W	W		Hap12
WK354	FS5202	tissue	ONCFS	F	5,20964	47,88620	F	1996	0.982	Wc	1	Wc	0.99965	F	W		Hap53
WK355	FS5203	tissue	ONCFS	F	5,20964	47,88620	F	1996	0.997	Wc	1	Wc	1	F	D	-	Hap49
WK356	FS5211	tissue	ONCFS	F			F	1996	0.997	Wc	1	Wc	0.99999	F	W	-	Hap03
WK357	FS5213	tissue	ONCFS	F			M	env 1996	0.98	Wc	1	Wc	0.99784	W	W		Hap03
WK358	FS5214	tissue	ONCFS	F	4,92799	48,03015	F	1997	0.995	Wc	1	Wc	0.99994	F	W		Hap12
WK359	FS5215	tissue	ONCFS	F	5,13697	47,98457	F	1997	0.998	Wc	1	Wc	1	F	W		Hap03
WK360	FS5401	tissue	ONCFS	F	5,89331	48,58280	M	1996	0.989	Wc	1	Wc	0.9998	D	W	-	Hap03
WK361	FS5402	tissue	ONCFS	F	5,89728	48,52200	M	1996	0.999	Wc	1	Wc	1	W	W		Hap03
WK362	FS5405	tissue	ONCFS	F	6,55874	48,52532	M	1997	0.993	Wc	1	Wc	0.99961	W	W		Hap03
WK363	FS5407	tissue	ONCFS	F	6,53808	48,72973	F	1997	0.996	Wc	1	Wc	1	F	W		Hap03
WK364	FS5408	tissue	ONCFS	F			F	1998	0.999	Wc	1	Wc	1	F	W		Hap05
WK365	FS54A	tissue	ONCFS	F	6,01937	48,66376	F	2005	0.949	Wc	1	Wc	0.96155	F	W		Hap03
WK366	FS54B	tissue	ONCFS	F	5,98617	48,83952	M	2005	0.747	BxW	1	BxW	0.99213	D	W	D	Hap03
WK367	FS54C	tissue	ONCFS	F	6,64801	48,57347	M	2005	0.998	Wc	1	Wc	1	W	W	W	Hap03
WK368	FS54D	tissue	ONCFS	F	6,11453	48,69343	F	2005	0.991	Wc	1	Wc	0.99991	F	W		Hap03
WK369	FS54E	tissue	ONCFS	F	5,92213	48,88888	M	2005	0.998	Wc	1	Wc	1	W	W		Hap03
WK370	FS5501	tissue	ONCFS	F	5,10970	48,71080	M	NA	0.998	Wc	1	Wc	1	W	W		Hap03
WK374	FS55D	tissue	ONCFS	F	5,50729	48,83832	M	2006	0.796	BxW	1	BxW	0.52805	W	W	W	Hap03
WK375	FS55E	tissue	ONCFS	F	5,03712	48,90460	F	2006	0.99	Wc	1	Wc	0.99997	F	W		Hap03
WK376	FS5701	tissue	ONCFS	F			M	NA	0.946	Wc	1	Wc	0.96283	W	W		Hap03
WK377	FS5702	tissue	ONCFS	F	6,37083	49,39479	M	1996	0.953	Wc	1	Wc	0.96719	W	W		Hap03
WK378	FS5703	tissue	ONCFS	F			F	env 1996	0.993	Wc	1	Wc	0.99996	F	W		Hap03
WK379	FS5704	tissue	ONCFS	F	6,37082	49,39478	M	1996	0.96	Wc	1	Wc	0.97993	W	W		Hap05
WK380	FS5705	tissue	ONCFS	F	6,36259	49,19715	M	1997	0.989	Wc	1	Wc	0.99991	W	W		Hap03
WK381	FS5708	tissue	ONCFS	F	6,44138	48,86696	M	1995	0.936	Wc	1	Wc	0.76958	W	W		Hap03
WK382	FS57A	tissue	ONCFS	F	7,44321	49,10428	M	2004	0.993	Wc	1	Wc	0.9999	W	W		Hap04
WK383	FS57B	tissue	ONCFS	F	6,93444	48,78460	F	2003	0.997	Wc	1	Wc	0.99999	F	W		Hap03

WK384	FS57C	tissue	ONCFS	F	6,90374	48,76057	M	2004	0.997	Wc	1	Wc	0.99999	W	W		Hap05
WK385	FS57D	tissue	ONCFS	F	6,90374	48,76057	M	2004	0.729	BxW	0.9791	BxW	0.63666	W	D	W	Hap07
WK386	FS57E	tissue	ONCFS	F	7,52088	48,98337	M	2006	0.971	Wc	1	Wc	0.99456	W	W		Hap03
WK387	FS5876	tissue	ONCFS	F	3,27336	47,23581	M	1996	0.998	Wc	1	Wc	1	W	W		Hap03
WK388	FS5902	tissue	ONCFS	F	4,20752	50,06668		2004	0.999	Wc	1	Wc	1	W	W		Hap03
WK389	FS6002	tissue	ONCFS	F	2,90089	49,61517	M	2000	0.908	Wc	0.99907	BxWxW	0.98486	W	W	-	Hap06
WK390	FS6302	tissue	ONCFS	F	3,08788	46,07274	F	1999	0.003	Dc	1	Dc	1	F	D		Hap08
WK391	FS6303	tissue	ONCFS	F	2,91509	45,72069	F	2000	0.943	Wc	1	Wc	0.82168	F	W		Hap05
WK392	FS6306	tissue	ONCFS	F	2,91507	45,72068	M	2000	0.812	BxW	0.99977	BxWxW	0.98327	W	W	-	Hap03
WK393	FS63A	tissue	ONCFS	F	2,96775	45,65930	F	2006	0.919	Wc	0.99995	BxWxW	0.84655	F	W		Hap03
WK394	FS64A	tissue	ONCFS	F	-,46086	42,99481	M	2004	0.998	Wc	1	Wc	1	W	W		Hap03
WK395	FS6703	tissue	ONCFS	F	7,47912	48,23699	M	1996	0.97	Wc	1	Wc	0.99212	F	W	-	Hap03
WK396	FS6711	tissue	ONCFS	F	7,61045	48,35485	F	1998	0.998	Wc	1	Wc	1	W	W		Hap03
WK397	FS6714	tissue	ONCFS	F	7,76997	48,85713	F	2000	0.998	Wc	1	Wc	1	F	W		Hap04
WK398	FS6723	tissue	ONCFS	F	7,61047	48,35485	M	2002	0.007	Dc	1	Dc	0.99996	D	D		Hap09
WK399	FS6724	tissue	ONCFS	F	7,71724	48,37397	M	2003	0.002	Dc	1	Dc	1	D	D		Hap10
WK400	FS6725	tissue	ONCFS	F			M	avant 2003	0.072	Dc	0.99999	Dc	0.95666	D	D		Hap07
WK401	FS6726	tissue	ONCFS	F	7,70454	48,41119	F	2003	0.02	Dc	1	Dc	0.99965	F	D		Hap08
WK402	FS6729	tissue	ONCFS	F	7,63593	48,37118	M	2004	0.004	Dc	1	Dc	0.99999	D	D		Hap07
WK404	FS67E	tissue	ONCFS	F	7,53307	48,76872	M	2005	0.002	Dc	1	Dc	1	D	D		Hap09
WK405	FS67G	tissue	ONCFS	F	7,63595	48,37119	M	2006	0.035	Dc	1	Dc	0.99706	D	D		Hap09
WK406	FS67I	tissue	ONCFS	F	7,30518	48,81996	M	2005	0.966	Wc	1	Wc	0.99719	W	W		Hap03
WK407	FS6801	tissue	ONCFS	F	7,32844	48,21800	M	1994	0.047	Dc	1	Dc	0.99497	F	D		Hap09
WK408	FS6805	tissue	ONCFS	F	7,15288	47,61299	M	1999	0.999	Wc	1	Wc	1	W	W		Hap03
WK409	FS6813	tissue	ONCFS	F	7,03746	47,71113	F	2003	0.003	Dc	1	Dc	1	F	D		Hap07
WK410	FS6904	tissue	ONCFS	F			F	env 1999	0.997	Wc	1	Wc	0.99999	F	W		Hap12
WK411	FS6905	tissue	ONCFS	F			M	env 1999	0.934	Wc	1	BxWxW	0.65559	W	W	-	Hap03
WK412	FS7703	tissue	ONCFS	F	3,24034	48,64801	F	1996	0.969	Wc	1	Wc	0.99791	F	W		Hap12
WK415	FS8802	tissue	ONCFS	F	6,17631	48,08879	M	1996	0.998	Wc	1	Wc	1	W	W		Hap12
WK416	FS8804	tissue	ONCFS	F	6,06904	47,51944	M	1997	0.009	Dc	1	Dc	0.99994	D	D		Hap14
WK417	FS8805	tissue	ONCFS	F	5,70847	48,15083	F	1997	0.957	Wc	1	Wc	0.99211	F	W		NA
WK418	FS8806	tissue	ONCFS	F	6,37501	48,15931	M	1997	0.003	Dc	1	Dc	1	D	D		Hap07

WK419	FS8808	tissue	ONCFS	F	6,61006	48,25181	M	1999	0.948	Wc	1	Wc	0.88031	W	W		Hap03
WK421	FS8813	tissue	ONCFS	F	5,88011	48,18253	F	2001	0.985	Wc	1	Wc	0.9997	W	W	-	Hap03
WK422	FS8814	tissue	ONCFS	F			F	avant 2002	0.935	Wc	1	BxWxW	0.50842	F	W		Hap03
WK423	FS88A	tissue	ONCFS	F	6,88287	48,36736	M	2005	0.998	Wc	1	Wc	1	W	W		Hap03
WK424	FS88B	tissue	ONCFS	F	6,96477	48,06845	F	2005	0.96	Wc	1	Wc	0.99479	F	W		Hap03
WK425	FS88C	tissue	ONCFS	F	6,30900	48,38735	F	2005	0.994	Wc	1	Wc	0.99998	F	W		Hap03
WK426	FS8902	tissue	ONCFS	F	3,65964	47,62813		env 1991	0.966	Wc	1	Wc	0.99616	W	W		Hap03
WK427	FS8907	tissue	ONCFS	F	3,75333	47,74648	M	1998	0.995	Wc	1	Wc	0.99998	W	D	-	Hap09
WK428	FS8908	tissue	ONCFS	F	3,67405	47,61608	M	1999	0.996	Wc	1	Wc	0.99999	W	W		Hap03
WK429	FS8909	tissue	ONCFS	F	3,47208	47,51871	F	1999	0.975	Wc	1	Wc	0.99276	F	D		Hap15
WK430	FS89B	tissue	ONCFS	F	3,67406	47,61609	M	2001	0.999	Wc	1	Wc	1	W	W		Hap12
WK431	FS89E	tissue	ONCFS	F	3,35516	47,73866	M	2004	0.926	Wc	1	BxWxW	0.76674	W	D	W	Hap09
WK432	FS89F	tissue	ONCFS	F	3,60692	47,92684	M	2005	0.004	Dc	1	Dc	1	D	D		Hap16
WK433	FS89G	tissue	ONCFS	F	3,53736	48,10839	M	2005	0.003	Dc	1	Dc	1	D	D		Hap16
WK434	FS89H	tissue	ONCFS	F	3,15442	47,68904	M	2005	0.947	Wc	1	Wc	0.90485	W	W		Hap04
WK435	FS89I	tissue	ONCFS	F	3,55264	47,83476	F	2005	0.994	Wc	1	Wc	0.99999	F	W		Hap03
WK436	FS89J	tissue	ONCFS	F	3,45699	47,94996	M	2005	0.997	Wc	1	Wc	1	W	W		Hap03
WK437	FS89K	tissue	ONCFS	F	3,62815	47,76000	M	2005	0.998	Wc	1	Wc	1	W	W		Hap03
WK438	FS89L	tissue	ONCFS	F	3,63425	48,07107	M	2005	0.999	Wc	1	Wc	1	W	D	W	Hap09
WK439	FS89MA	tissue	ONCFS	F	4,09564	47,53497	M	2005	0.08	Dc	0.99999	Dc	0.97609	D	D		Hap14
WK440	FS89MB	tissue	ONCFS	F	3,55793	47,83215	M	2005	0.005	Dc	1	Dc	0.99999	D	D		Hap09
WK441	FS89N	tissue	ONCFS	F	3,40927	47,99789	M	2004	0.002	Dc	1	Dc	1	D	D		Hap08
WK442	FSC01A	tissue	ONCFS	F	4,92669	45,94858	F	2004	0.002	Dc	1	Dc	1	F	D		Hap09
WK443	FSC1005	tissue	ONCFS	F	4,24736	48,21105	F	2005	0.018	Dc	1	Dc	0.99973	F	D		Hap09
WK444	FSC67B	tissue	ONCFS	F	7,47911	48,23698	M	2005	0.004	Dc	1	Dc	0.99998	D	D		Hap09
WK445	FSC89C	tissue	ONCFS	F	3,60934	47,83349	F	2004	0.007	Dc	1	Dc	0.99996	F	D		Hap08
WK446	FSC89D	tissue	ONCFS	F			F	2004	0.002	Dc	1	Dc	1	F	D		Hap09
WK447	FSCD51	tissue	ONCFS	F	7,30349	48,49715	F	1999	0.016	Dc	1	Dc	0.99996	F	D		Hap16
WK448	FSGEX01	tissue	ONCFS	F	6,05052	46,36980	F	2005	0.003	Dc	1	Dc	1	D	D		Hap17
WK449	FS0102	hair	ONCFS	F	5,15364	46,25615	M	1998	0.997	Wc	1	Wc	0.99998	W	W		NA
WK450	FS0201	hair	ONCFS	F	3,76959	49,57941	M	1998	0.97	Wc	1	Wc	0.99588	W	W		NA
WK451	FS0202	hair	ONCFS	F	3,83626	49,50642	M	1999	0.95	Wc	1	Wc	0.94389	W	W		NA
WK452	FS0304	hair	ONCFS	F	2,69266	46,59033	M	1996	0.996	Wc	1	Wc	1	W	W		NA

WK453	FS0305	hair	ONCFS	F	2,61167	46,53407	M	1997	0.929	Wc	0.99999	BxWxW	0.57793	W	D	-	Hap09
WK454	FS0306	hair	ONCFS	F	3,05012	46,28593	M	env 1993	0.808	BxW	1	BxWxW	0.53266	D	W	-	Hap03
WK455	FS0307	hair	ONCFS	F	3,05012	46,28593	F	env 1994	0.985	Wc	1	Wc	0.99742	F	W		Hap03
WK456	FS0308	hair	ONCFS	F	3,05012	46,28593	F	env 1994	0.999	Wc	1	Wc	1	F	W		Hap03
WK457	FS0309	hair	ONCFS	F	3,05012	46,28593	F	env 1995	0.91	Wc	0.99996	BxWxW	0.87111	F	W		NA
WK458	FS1827	hair	ONCFS	F	2,99508	46,97659	M	1997	0.003	Dc	1	Dc	1	D	D		NA
WK459	FS1828	hair	ONCFS	F	2,35085	46,89508	M	1996	0.996	Wc	1	Wc	0.99999	W	W		NA
WK460	FS2302	hair	ONCFS	F	2,28470	46,17605	F	env 1995	0.916	Wc	0.99999	BxWxW	0.52421	F	W		NA
WK461	FS2303	hair	ONCFS	F	1,84882	46,15399	F	env 1995	0.998	Wc	1	Wc	1	F	D		NA
WK462	FS2304	hair	ONCFS	F	2,04977	46,35791	M	1998	0.902	Wc	0.99721	BxWxW	0.99098	D	W	-	NA
WK464	FS3615	hair	ONCFS	F	1,17182	46,80975	M	1996	0.992	Wc	1	Wc	0.99996	W	W		NA
WK465	FS3617	hair	ONCFS	F			M	env 1995	0.009	Dc	1	Dc	0.99995	D	D		NA
WK467	FS3619	hair	ONCFS	F	1,48522	46,77887	M	1997	0.993	Wc	1	Wc	0.99995	W	W		NA
WK468	FS3620	hair	ONCFS	F	1,54600	46,79823	F	1997	0.997	Wc	1	Wc	0.99999	F	D		Hap09
WK469	FS3621	hair	ONCFS	F	1,78695	46,70579	M	1996	0.971	Wc	1	Wc	0.99796	W	W		Hap05
WK470	FS3622	hair	ONCFS	F	1,31199	46,72161	F	1997	0.997	Wc	1	Wc	0.99999	F	W		Hap05
WK471	FS3623	hair	ONCFS	F	2,07945	46,49189	M	1996	0.47	F1	0.99993	F1	0.99984	F	D	-	Hap09
WK472	FS3916	hair	ONCFS	F	5,60659	47,19773	M	2003	0.998	Wc	1	Wc	0.99999	W	W		Hap03
WK473	FS4104	hair	ONCFS	F			M	1997	0.004	Dc	1	Dc	0.99999	D	D		NA
WK474	FS4105	hair	ONCFS	F	1,48880	47,44255	M	1998	0.943	Wc	1	Wc	0.89058	D	W	-	NA
WK475	FS4106	hair	ONCFS	F	2,17853	47,51776	M	1998	0.998	Wc	1	Wc	1	D	W	D	NA
WK476	FS5204	hair	ONCFS	F			F	1997	0.002	Dc	1	Dc	1	F	D		NA
WK477	FS5710	hair	ONCFS	F	6,95219	49,07939	F	1996	0.948	Wc	1	Wc	0.94673	F	W		Hap03
WK478	FS5711	hair	ONCFS	F	7,52134	48,97024	F	env 1995	0.977	Wc	1	Wc	0.99859	F	W		NA
WK479	FS7704	hair	ONCFS	F	2,83888	48,43327	M	1996	0.267	BxD	1	BxD	0.99929	D	W	D	Hap03
WK480	FS7705	hair	ONCFS	F	2,96905	48,21679	M	1996	0.991	Wc	1	Wc	0.99996	W	W		Hap03
WK481	FS7706	hair	ONCFS	F			M	1997	0.998	Wc	1	Wc	1	W	W		NA

WK482	FS8807	hair	ONCFS	F			M	env 1995	0.978	Wc	1	Wc	0.99721	W	W		NA
WK483	FSCD50	hair	ONCFS	F	7,71725	48,37398	M	1999	0.003	Dc	1	Dc	1	D	D		NA
WK484	FSCD54	hair	ONCFS	F	7,74676	48,44039	F	1999	0.008	Dc	1	Dc	0.9999	F	D		NA
WK485	FSX0	hair	ONCFS	F			M	NA	0.998	Wc	1	Wc	1	W	W		Hap12
Dc52	506/162A 01.05.2010	Single hair	WKM	CH	6.21175	46.60265		2010	0.002	Dc	1	Dc	1	D	D		Hap32
Dc53	506/162E 01.05.2010	1hair	WKM	CH	6.21175	46.60265		2010	0.005	Dc	1	Dc	0.99999	D	D		Hap09
Dc56	511/122G 08.12.2009	1hair	WKM	CH	6.28468	46.24355		2009	0.002	Dc	1	Dc	1	D	D		Hap09
Dc38	512/146C 01.01.2010	1hair	WKM	CH	6.29310	46.45955		2010	0.002	Dc	1	Dc	1	D	D		Hap09
ID08	512/154A 01.04.2010	1hair	WKM	CH	6.29158	46.53151		2010	0.999	Wc	1	Wc	1	W	W	-	Hap12
ID09	512/154A 18.04.2010	1hair	WKM	CH	6.29158	46.53151		2010	0.997	Wc	1	Wc	0.99999	F	W	-	Hap12
ID13	515/158B 19.04.2009	1hair	WKM	CH	6.32994	46.56787		2009	0.998	Wc	1	Wc	1	W	W	-	Hap12
Dc10	518/162A 16.01.2010	1hair	WKM	CH	6.36835	46.60423		2010	0.002	Dc	1	Dc	1	D	D		Hap16
Dc39	518/162C 01.01.2010	1hair	WKM	CH	6.36835	46.60423		2010	0.002	Dc	1	Dc	1	D	D		Hap28
Dc25	527/166A 07.03.2009	1hair	WKM	CH	6.48518	46.64125		2009	0.033	Dc	1	Dc	0.99446	D	D		Hap09
Dc26	527/166A 07.03.2009	1hair	WKM	CH	6.48518	46.64125		2009	0.008	Dc	1	Dc	0.9999	D	D		Hap14
Dc47	530/186C 01.04.2010	1hair	WKM	CH	6.52130	46.82147		2010	0.003	Dc	1	Dc	1	D	D		Hap22
Dc15	530/194B 04.05.2010	1hair	WKM	CH	6.52008	46.89343		2010	0.018	Dc	1	Dc	0.99967	D	D		Hap16
Dc22	533/198A 04.02.2009	1hair	WKM	CH	6.55885	46.92971		2009	0.004	Dc	1	Dc	0.99999	F	D		Hap21
Dc55	536/194D 15.06.2010	1hair	WKM	CH	6.59880	46.89403		2010	0.023	Dc	1	Dc	0.99924	D	D		Hap19
Dc17	536/202B 01.06.2010	1hair	WKM	CH	6.59768	46.96599		2010	0.003	Dc	1	Dc	1	D	D		Hap08
Dc21	539/198C 21.01.2009	1hair	WKM	CH	6.63763	46.93029		2009	0.019	Dc	1	Dc	0.99983	D	D		Hap25
Dc49	545/214A 20.04.2010	1hair	WKM	CH	6.71448	47.07473		2010	0.018	Dc	1	Dc	0.99918	D	D		Hap08
Dc14	545/214B 20.04.2010	1hair	WKM	CH	6.71448	47.07473		2010	0.004	Dc	1	Dc	1	D	D		Hap09
Dc50	545/214B 20.04.2010	1hair	WKM	CH	6.71448	47.07473		2010	0.04	Dc	1	Dc	0.98921	D	D		Hap30
Dc16	554/202B 28.05.2010	1hair	WKM	CH	6.83419	46.96748		2010	0.003	Dc	1	Dc	1	D	D		Hap09
Dc09	560/218C 13.01.2010	1hair	WKM	CH	6.91161	47.11178		2010	0.005	Dc	1	Dc	0.99998	D	D		Hap09
Dc03	563/206C 26.12.2008	1hair	WKM	CH	6.95212	47.00402		2008	0.004	Dc	1	Dc	1	D	D		Hap21
ID10	566/210B 28.05.2010	1hair	WKM	CH	6.99127	47.04016		2010	0.999	Wc	1	Wc	1	W	W	W	Hap03
Dc19	566/218A 15.06.2010	1hair	WKM	CH	6.99067	47.11212		2010	0.007	Dc	1	Dc	0.99998	D	D		Hap22
Dc54	566/218B 02.06.2010	1hair	WKM	CH	6.99067	47.11212		2010	0.017	Dc	1	Dc	0.99971	D	D		Hap22
Dc18	566/218C 15.06.2010	1hair	WKM	CH	6.99067	47.11212		2010	0.003	Dc	1	Dc	1	D	D		Hap07
ID06	566/258C 20.02.2010	1hair	WKM	CH	6.98764	47.47189		2010	0.999	Wc	1	Wc	1	F	W	-	Hap03

Dc36	572/218A 29.12.2009	1hair	WKM	CH	7.06972	47.11240	2009	0.002	Dc	1	Dc	1	D	D		Hap17
Dc28	572/218B 15.12.2009	1hair	WKM	CH	7.06972	47.11240	2009	0.056	Dc	0.99998	Dc	0.97075	D	D		Hap17
Dc29	572/218C 15.12.2009	1hair	WKM	CH	7.06972	47.11240	2009	0.005	Dc	1	Dc	0.99997	D	D		Hap11
Dc30	572/218C 15.12.2009	1hair	WKM	CH	7.06972	47.11240	2009	0.005	Dc	1	Dc	0.99998	D	D		Hap09
Dc37	572/218C 29.12.2009	1hair	WKM	CH	7.06972	47.11240	2009	0.002	Dc	1	Dc	1	D	D		Hap11
HybID03	572/258A 20.02.2010	1hair	WKM	CH	7.06723	47.47218	2010	0.847	BxW	0.99898	BxWxW	0.9934	F	W	-	Hap12
ID14	572/258C 06.02.2010	1hair	WKM	CH	7.06723	47.47218	2010	0.998	Wc	1	Wc	1	W	W	-	Hap03
Dc48	578/226B 06.04.2010	1hair	WKM	CH	7.14838	47.18459	2010	0.005	Dc	1	Dc	0.99999	D	D		Hap09
Dc51	578/234A 20.04.2010	1hair	WKM	CH	7.14799	47.25654	2010	0.017	Dc	1	Dc	0.99988	F	D		Hap31
Dc13	578/234A 26.02.2010	1hair	WKM	CH	7.14799	47.25654	2010	0.003	Dc	1	Dc	1	D	D		Hap19
ID05	584/250C 21.01.2010	1hair	WKM	CH	7.22669	47.40062	2010	0.998	Wc	1	Wc	1	W	W	W	Hap03
Dc12	590/250B 07.01.2010	1hair	WKM	CH	7.30617	47.40074	2010	0.004	Dc	1	Dc	0.99999	D	D		Hap16
ID15	593/238B 19.01.2010	1hair	WKM	CH	7.34610	47.29285	2010	0.98	Wc	1	Wc	0.99459	W	W	-	Hap03
Dc31	593/252B 15.12.2009	1hair	WKM	CH	7.34588	47.41877	2009	0.024	Dc	1	Dc	0.99921	D	D		Hap07
Dc32	593/252B 15.12.2009	1hair	WKM	CH	7.34588	47.41877	2009	0.004	Dc	1	Dc	1	F	D		Hap07
ID04	596/242A 26.12.2009	1hair	WKM	CH	7.38572	47.32885	2009	0.999	Wc	1	Wc	1	W	W	W	Hap12
Dc42	596/242C 09.01.2010	1hair	WKM	CH	7.38572	47.32885	2010	0.005	Dc	1	Dc	0.99999	D	D		Hap09
ID02	596/250B 15.12.2009	1hair	WKM	CH	7.38565	47.40081	2009	0.996	Wc	1	Wc	0.99998	F	W	-	Hap33
Dc33	598/248C 15.12.2009	1hair	WKM	CH	7.41215	47.38283	2009	0.04	Dc	1	Dc	0.99646	D	D		Hap27
HybID02	598/248C 29.12.2009	1hair	WKM	CH	7.41215	47.38283	2009	0.741	BxW	1	BxW	0.99741	D	W	D	Hap33
ID01	599/246A 29.01.2009	1hair	WKM	CH	7.42540	47.36484	2009	0.985	Wc	1	Wc	0.99875	W	W	W	Hap03
ID11	599/254B 06.02.2009	1hair	WKM	CH	7.42538	47.43679	2009	0.995	Wc	1	Wc	0.99997	F	D	-	Hap23
ID12	599/254B 23.01.2009	1hair	WKM	CH	7.42538	47.43679	2009	0.981	Wc	1	Wc	0.99997	W	D	-	Hap09
HybID04	602/242B 07.01.2010	1hair	WKM	CH	7.46510	47.32886	2010	0.738	BxW	1	BxW	0.99877	D	W	-	Hap03
ID07	602/242B 20.02.2010	1hair	WKM	CH	7.46510	47.32886	2010	0.996	Wc	1	Wc	0.99999	W	W	W	Hap12
ID03	602/250C 15.12.2009	1hair	WKM	CH	7.46513	47.40082	2009	0.983	Wc	1	Wc	0.99946	W	D	W	Hap09
Dc45	605/238B 19.01.2010	1hair	WKM	CH	7.50474	47.29287	2010	0.003	Dc	1	Dc	1	D	D		Hap09
HybID01	605/246A 21.03.2009	1hair	WKM	CH	7.50483	47.36482	2009	0.724	BxW	1	BxW	0.99895	D	W	D	Hap03
Dc23	608/242A 04.02.2009	1hair	WKM	CH	7.54447	47.32882	2009	0.005	Dc	1	Dc	0.99998	D	D		Hap26
Dc02	608/242A 07.01.2009	1hair	WKM	CH	7.54447	47.32882	2009	0.009	Dc	1	Dc	0.99996	D	D		Hap14
Dc04	608/242A 21.01.2009	1hair	WKM	CH	7.54447	47.32882	2009	0.014	Dc	1	Dc	0.99987	D	D		Hap22
Dc01	608/242B 07.01.2009	1hair	WKM	CH	7.54447	47.32882	2009	0.011	Dc	1	Dc	0.9999	D	D		Hap20
Dc05	608/242B 21.01.2009	1hair	WKM	CH	7.54447	47.32882	2009	0.006	Dc	1	Dc	0.99997	D	D		Hap23
Dc24	608/242C 04.02.2009	1hair	WKM	CH	7.54447	47.32882	2009	0.005	Dc	1	Dc	0.99999	F	D		Hap09
Dc20	609/251A 20.01.2009	1hair	WKM	CH	7.55788	47.40975	2009	0.016	Dc	1	Dc	0.99998	D	D		Hap19

Dc34	613/255B 15.12.2009	1hair	WKM	CH	7.61099	47.44566	2009	0.021	Dc	1	Dc	0.9988	F	D	Hap19
Dc35	613/256C 15.12.2009	1hair	WKM	CH	7.61102	47.45466	2009	0.003	Dc	1	Dc	1	F	D	Hap09
Dc08	614/234C 08.01.2010	1hair	WKM	CH	7.62359	47.25676	2010	0.002	Dc	1	Dc	1	D	D	Hap09
Dc41	614/242B 08.01.2010	1hair	WKM	CH	7.62384	47.32872	2010	0.004	Dc	1	Dc	1	D	D	Hap09
Dc06	614/250A 06.12.2009	1hair	WKM	CH	7.62409	47.40067	2009	0.05	Dc	1	Dc	0.98577	D	D	Hap09
Dc40	614/258A 03.01.2010	1hair	WKM	CH	7.62434	47.47262	2010	0.006	Dc	1	Dc	0.99999	D	D	Hap22
Dc46	620/242A 22.01.2010	1hair	WKM	CH	7.70322	47.32856	2010	0.038	Dc	1	Dc	0.99102	F	D	Hap28
Dc27	632/242B 06.12.2009	1hair	WKM	CH	7.86196	47.32809	2009	0.002	Dc	1	Dc	1	F	D	Hap09
Dc11	632/242B 16.01.2010	1hair	WKM	CH	7.86196	47.32809	2010	0.002	Dc	1	Dc	1	D	D	Hap09
Dc43	632/250C 12.01.2010	1hair	WKM	CH	7.86253	47.40004	2010	0.004	Dc	1	Dc	0.99999	D	D	Hap29
Dc44	644/250B 13.01.2010	1hair	WKM	CH	8.02149	47.39935	2010	0.011	Dc	1	Dc	0.99993	D	D	Hap07
Dc07	644/250B 13.01.2010	1hair	WKM	CH	8.02149	47.39935	2010	0.022	Dc	1	Dc	0.9993	D	D	Hap24
2-28		1hair	Blauen	CH	7.57129	47.47270	2006	0.002	Dc	1	Dc	1	D	D	Hap09
4-67dil		1hair	Blauen	CH	7.38560	47.45477	2007	0.013	Dc	1	Dc	0.99994	D	D	Hap08
6-55		1hair	Blauen	CH	7.50492	47.43678	2007	0.006	Dc	1	Dc	0.99997	D	D	Hap19
HK004	Grissini	hair	Hasler	CH	7.16199	47.10366	2005	0.006	Dc	1	Dc	0.99996	D	D	NA
HK054	Jack	hair	Bader	CH	7.22783	47.11280	2005	0.027	Dc	1	Dc	0.99834	D	D	NA
HK058		tissue	vet	CH	6.81949	47.10233	2006	0.002	Dc	1	Dc	1	F	D	Hap22
HK059		tissue	vet	CH	6.81949	47.10233	2006	0.004	Dc	1	Dc	0.99999	F	D	Hap34
HK060		tissue	vet	CH	6.81949	47.10233	2006	0.003	Dc	1	Dc	1	F	D	Hap09
HK064		tissue	vet	CH	6.81949	47.10233	2006	0.002	Dc	1	Dc	1	D	D	Hap08
HK065		tissue	vet	CH	6.81949	47.10233	2006	0.007	Dc	1	Dc	0.99994	D	D	Hap09
HK080		tissue	gamek.	CH	7.34619	47.23888	2011	0.004	Dc	1	Dc	1	D	D	Hap09
HK081		tissue	gamek.	CH	7.37254	47.29287	2011	0.013	Dc	1	Dc	0.99985	F	D	Hap09
HK082		tissue	gamek.	CH	7.37254	47.29287	2011	0.005	Dc	1	Dc	0.99999	F	D	Hap09
HK083		tissue	gamek.	CH	7.37254	47.29287	2011	0.027	Dc	1	Dc	0.99747	D	D	Hap09
HK084		tissue	gamek.	CH	7.37254	47.29287	2011	0.006	Dc	1	Dc	0.99999	F	D	Hap09
HK086		tissue	gamek.	CH	7.66323	47.25669	2011	0.002	Dc	1	Dc	1	F	D	Hap35
HK087		tissue	gamek.	CH	7.45184	47.23892	2011	0.004	Dc	1	Dc	1	F	D	NA
HK088		tissue	gamek.	CH	6.79304	47.11118	2011	0.002	Dc	1	Dc	1	F	D	Hap09
HK089		tissue	gamek.	CH	6.72752	47.08381	2011	0.004	Dc	1	Dc	0.99999	D	D	NA
HK092		tissue	Flückiger	CH	7.83564	47.34617	2011	0.008	Dc	1	Dc	0.99999	D	D	NA
Nuglar		1hair	Murer	CH	7.70393	47.47247	2011	0.008	Dc	1	Dc	0.99992	D	D	NA
1-02		1hair	Blauen	CH	7.45189	47.43679	2006	0.936	Wc	1	Wc	0.92433	F	D	Hap09
2-06		1hair	Blauen	CH	7.43864	47.44579	2006	0.998	Wc	1	Wc	1	W	D	- Hap09

2-35		1hair	Blauen	CH	7.38561	47.43678	2006	0.997	Wc	1	Wc	0.99999	F	W	Hap06
4-17		1hair	Blauen	CH	7.46515	47.44579	2006	0.909	Wc	0.99786	BxWxW	0.99711	W	D -	Hap19
5-73		1hair	Blauen	CH	7.57126	47.46370	2007	0.995	Wc	1	Wc	0.99991	F	W	Hap03
6-32		1hair	Blauen	CH	7.42538	47.45478	2007	0.996	Wc	1	Wc	0.99999	W	W	Hap12
6-41		1hair	Blauen	CH	7.57122	47.44571	2007	0.995	Wc	1	Wc	0.99995	F	D	Hap09
Dupre		1hair	gamek.	CH	6.83328	47.04843	2012	0.998	Wc	1	Wc	1	F	W	Hap12
4-09		1hair	Blauen	CH	7.37235	47.44577	2006	0.83	BxW	0.99999	BxWxW	0.974	W	W	Hap18
6-53dil		1hair	Blauen	CH	7.39886	47.44578	2007	0.886	Wc	0.71133	BxWxW	0.99959	F	D	Hap09
5-110d		1hair	Blauen	CH	7.57124	47.45471	2007	0.243	BxD	0.99999	BxD	0.97877	D	W	Hap05
5-111		1hair	Blauen	CH	7.57124	47.45471	2007	0.201	BxD	0.99871	BxD	0.73559	F	D	Hap09
WK486	Steffen11b	hair	Stefen	D	12.26082	50.47811	2011	0.997	Wc	1	Wc	1	W	W	NA
WK487	Steffen12a	hair	Stefen	D			2012	0.058	Dc	1	Dc	0.99844	D	D	NA
WK488	Vu13_010410_12_37	hair	Patry	F	5.64615	45.63922	2010	0.017	Dc	1	Dc	0.99958	D	D	NA
WK489	Vuache10_040310_7_62	hair	Patry	F	5.64959	45.63635	2010	0.994	Wc	1	Wc	1	F	W	NA

Perspectives

In this thesis, I first established a method to discover diagnostic SNP-markers, using reduced representation libraries of wildcat and domestic cat genomes and next-generation sequencing of these libraries. This methodological approach is likely to be useful for discovering diagnostic markers in other hybridizing species. Further, I found 200 SNP-markers which allow assessing individual levels of introgression in wildcats and domestic cats. This will facilitate future more in depth studies of wildcats, domestic cats and their hybrids. I then optimized a SNP-genotyping method to be usable for low quality/quantity nuclear DNA. This expands the field of usability of non-invasively collected samples, like faeces or single hairs.

I applied these technical advances to a large dataset of wildcats, domestic cats and hybrids, principally from Switzerland, France and Germany, which led to the following new findings about European wildcats. The wildcat population in the Swiss Jura had 2% domestic migrants per generation. A same migration rate was found based on the wildcat dataset from France, Germany and Switzerland. In contrast, the rate of introgression from wildcats into free-ranging domestic cats was lower (0.4%). A migration rate of 2% seems relatively low, but still could be sufficient to threaten the wildcat from genetic extinction under certain – rather unrealistic – demographic conditions, like constant migration rate at each generation and constant population size. Further, in wildcats, the maternal line is more often introgressed with domestic cats than the paternal line. This is not necessarily due to more matings between wildcat males and domestic females than vice versa and is most likely explained by male-biased dispersal. Moreover, hybrids seem to be mostly distributed at local edges of wildcat populations and the wildcat population in the Franco-Swiss Jura may be growing. These observed hybridization patterns might be explained by a selectively neutral model involving an expansion of the wildcat populations.

Further research

The findings of this thesis should be completed by further analyses of introgression patterns. Especially, it would be worth studying introgression at a broader spatio-temporal scale and with more demographic parameters, like effective population sizes or fitness of parental populations and their hybrids. Here, I sketch a few issues and questions that might be relevant to address for a better understanding of the mechanisms leading to introgression.

Spatial pattern of hybridization

Is hybridization mainly located at distribution edges?

How does demography of humans, domestic cats and wildcats influence the spatial hybridization pattern?

Hybridization between wildcats and domestic cats is widespread, but seems to be located at local edges of wildcat distribution and often near to cities, at least in the region of the Franco-German-Swiss borders (Figure 1 of chapter 4). Such an edge effect has also been observed in wildcats in Italy (Randi 2008), as well as in wolves hybridizing with domestic dogs (Verardi et al. 2006; Godinho et al. 2011) and between an endemic subspecies of red fox and an introduced red fox population (Sacks et al. 2011). However, more in depth quantitative spatial analyses are still needed. The wildcat distribution should be assessed at smaller scale to define distribution edges more accurately. It would also be worth analyzing interactions between human densities and wildcat hybrid densities. Human densities would act as proxy for stray/domestic cat densities. In addition, human settlements may cause wildcat habitat fragmentation. Small fragmented wildcat populations might be more subjected to introgression than geographically large and continuous populations, because they lack a stable source population. Further, geographic hybridization patterns seem to differ between the Franco-Swiss-German region and for example Hungary or Scottland, where introgression seems to be omnipresent and not limited to the edges (Beaumont et al. 2001; Lecis et al. 2006; Randi 2008). It would be important to understand how these spatial differences arise and how differences in permeability of reproductive barriers within a same subspecies evolve. Differences in demography, colonization history and ecology may play important roles. For example, habitat, climate or human culture of keeping pets in Hungary and Scottland may favor higher density of stray or feral cats than in other countries. Moreover, in Hungary, introgression may be increased by natural hybridization with African wildcats *(Felis silvestris libyca)*. The African wildcat may be increasingly occurring further north than its supposed distribution (Driscoll et al. 2007), due to climate warming.

Temporal pattern of hybridization

Is introgression a time-limited process?

Introgression is likely to increasingly threaten the genetic integrity of a species if ongoing over a longer time period. It is thus crucial to assess the time scale of introgression. Looking back in time is difficult, even if several historical samples would be available in natural history museums (Wandeler et al. 2007). In our historical dataset of 19 cats from 1915 to 1990, we found three wildcats with introgressed domestic mitochondrial haplotype (dating from 1973 in Asuel, Jura; 1988 Alle, Jura and 1989 Péry, Berne) and one backcross into wildcat (dated 1981, in Gänsbrunnen, Solothurn), suggesting – not very surprisingly – that introgression already occurred in the 1970ies. However, it is difficult to get an accurate estimation of introgression rates in historical cat populations based only on museum samples, because sampling effort and bias of a hundred year ago may not be comparable to the ones of present day studies. For example, historic samples are more often hunted animals, thus the sampled habitat might be different from today, where most of the samples are road kills. In addition, historic samples not matching perfectly with the wild phenotype, i.e. potential hybrids, may not have survived the successive clearance of museum collections over the years. Thus, looking forward is probably the better strategy to infer changes in introgression over time. Monitoring wildcat

populations at regular time intervals, e.g. all nine years (roughly three cat generations), with similar sampling strategy and effort as done in the present study, would help disclose a temporal hybridization pattern. The temporal pattern should be inferred together with the demographic and spatial hybridization patterns, since it is likely that all these factors are linked.

Ancient DNA

What is the genome of a “pure” wildcat?

Defining reference wildcats is difficult nowadays, since domestic cats are sympatric with wildcats for more than 2000 years and all modern wildcats might already be introgressed. European wildcat may be genetically defined more precisely by analyzing ancient wildcat samples, from about 2500 years ago, i.e. before domestic cats were present in the habitat of European wildcats. However, with the approach used in the present thesis, the markers could not be developed in ancient samples (see discussion in chapter 1). Nevertheless, the SNP-genotyping technique developed here for low quality/quantity DNA samples may be applicable to ancient DNA as well. Thus, it may now be possible to confirm the validity of the diagnostic SNP-markers by analyzing ancient samples. On the other hand, it is very likely that the markers, which are still almost diagnostic nowadays, were already diagnostic 2500 years ago.

Hybrid fitness

Do hybrids suffer from outbreeding depression?

Introgressed individuals may have severely reduced fitness in comparison to non-introgressed ones (Muhlfeld et al. 2009; Alund et al. 2013). However, we did not find evidence for reduced fitness in wildcat hybrids. The ratio between first, second and third generation hybrids was 1:10:13, which is even more than the expected 1:2:4 in a population of constant size (but see discussion in chapter 3). Further, the ratio of juveniles and adults found in the dataset is the same for introgressed and non-introgressed cats. Body mass might be a somewhat unreliable proxy for fitness, especially if collection month is unknown, since body mass of wildcats undergo dramatic seasonal changes (between 1.5 and 2.5kg for males and 0.250-2.15kg for females) and even daily changes (up to 0.5kg; Condé & Schauenberg 1969; Raydelet 2009). However, more detailed data on parasitic load or body measures from most wildcat road kills found in Switzerland would be available in the autopsy reports from the Centre for Fish and Wildlife Health of Berne, who collected the carcasses. In addition, telemetry studies with individuals of known introgression level may reveal reproductive success of hybrids, e.g. by counting offspring of collared females or by analyzing survival rate of collared hybrids.

Speciation genes

Which genomic regions differentiate wildcats and domestic cats?

Wildcat hybrids seem to keep the wild tabby phenotype, despite nuclear introgression with domestic cats (see Figure 2 in the introduction, p. 27). This observation is likely biased by the fact that hybrids which do not exhibit the wild phenotype do not get sampled. Nevertheless, hairs of backcrosses sampled for chapter 3 also tended to have similar colors to wildcat hairs, i.e. they were mostly tabby. Hence, the wild phenotype might be under strong positive selection (Currat et al. 2008). Genes for this phenotype may be significantly less introgressed than neutral genes. Genomic studies of introgressed individuals may reveal the genes favoring speciation between both subspecies. Using a genomic approach, Emelianov et al. (2004) found a strong heterogeneity of molecular divergence between the strongly differentiated larch and pine-feeding host races of *Zeiraphera diniana* in areas where hybridization occurs. They suggest that sympatric differentiation is maintained by selection, but hybridization homogenizes much of the genetic variation in neutral genomic regions. Obviously, such genes may not only concern the phenotype but also behavioural or physiological differences. When comparing wildcats to domestic cats, genes involved in domestication might be discovered.

Conservation measures

Is introgression with domestic cats a chance or a risk for the wildcats? I couldn't answer this main conservation question which triggered my PhD thesis. "It is an understatement to say that hybridization is a complex business!" This sentence of Graham Stone (2000) emphasizes that the way to a satisfying answer may still be long. At the two extremes, introgression can lead to adaptive evolution or to genetic extinction. In the middle, introgression may simply be a time-limited side effect of demographic changes (Currat et al. 2008). In this thesis, we found evidence for the latter, neutral explanation. However, it would be an overhasty conclusion to state that introgression is not a risk to wildcats, since many important aspects, e.g. demography, ecology and time, were not sufficiently considered so far.

Nevertheless, the methods to detect hybrids presented in this thesis may be useful for many practical aspects of wildcat conservation. For example, they may identify which individuals should be involved in behavioral studies focusing on hybridization. They can also help managers of breeding programs or of reintroduction projects to decide on which individuals to breed or release. In addition, the level of introgression in free-ranging wildcats estimated in this thesis may be used as a baseline for future analyses of introgression on a broader spatio-temporal scale.

In the meantime, based on the precautionary principle, introgression should still be considered as relevant to species conservation. I believe a key conservation goal in this respect should be the knowledge of the mechanisms leading to introgression. Thus, the main conservation measures should now be to monitor wildcat populations over time in respect to their introgression level, e.g. every 9-12 years (three-four cat generations) and to promote and support further research about wildcat introgression.

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